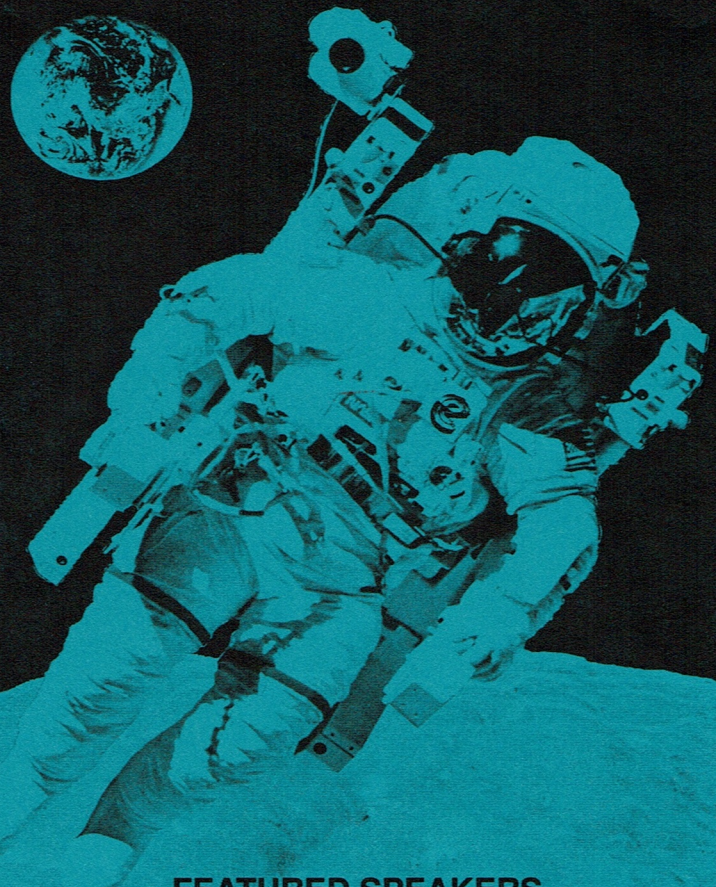
# VITAMIN C

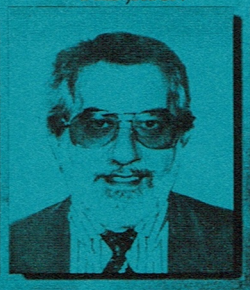
## *The future is now!*

**Saturday, March 11, 5:30-6:30 pm**
**Anaheim Hilton–Ballroom A**

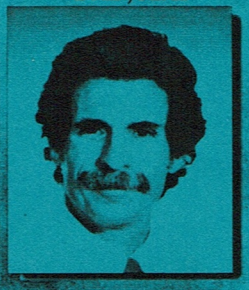


### FEATURED SPEAKERS

Jeffrey Bland, PhD

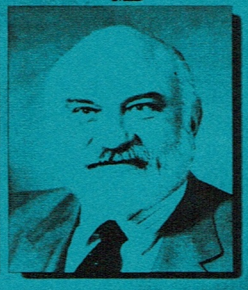


Anthony Verlangieri, PhD,FACN



Steven Silverstein, DMD, MPH

Richard Passwater, PhD



Hugh Riordan, MD

L. Phillips Brown, DVM

**Sponsored by Inter-Cal Corporation, exclusive manufacturer of Ester-C® Calcium, Magnesium, Zinc, Potassium and Sodium Ascorbates.**

Ester-C® and the e-c logo are licensed trademarks of Inter-Cal Corp.

**REFRESHMENTS, WINE AND HORS D' OUEVRES WILL BE SERVED**

ESTER-C®

ISSN 0306-3623

# General Pharmacology

Volume 25 Number 7 November 1994

## Contents


| | | |
|---|---|---|
| Michael J. Fay, Marilyn J. Bush and Anthony J. Verlangieri | 1465 | Effect of aldonic acids on the uptake of ascorbic acid by 3T3 mouse fibroblasts and human T lymphoma cells |
| Z. Sevim Ercan, R. Kazim Türker, Canan Uluoglu, Özlem Yazar and Hakan Zengil | 1471 | The effects of endothelin peptides in the rabbit isolated perfused kidney |
| Katsuo Koike, Takahiro Horinouchi and Issei Takayanagi | 1477 | Comparison of interactions of R(+)- and S(−)-isomers of β-adrenergic partial agonists, befunolol and carteolol, with high affinity site of β-adrenoceptors in the microsomal fractions from guinea-pig ciliary body, right atria and trachea |
| Simon W. Rabkin, Nicholas Freestone and Gary A. Quamme | 1483 | Nifedipine does not alter the increased cystolic free magnesium during inhibition of mitochondrial function in isolated cardiac myocytes |
| Halim Soncul, Oğuz Tatlıcan, Velit Halit, Eser Öz, Volkan Sinci, Ergun Salman, Levent Gökgöz, Nurten Türkozkan and Ali Ersöz | 1493 | The effect of selenium added cardioplegia in guinea pigs |
| Güler Öztürk, Deniz Erbas, Turgut İmir and Naci, M. Bor | 1499 | Decreased natural killer (NK) cell activity in zinc-deficient rats |
| Ayman O. S. El-Kadi and Suleiman I. Sharif | 1505 | The influence of various experimental conditions on the expression of naloxone-induced withdrawal symptoms in mice |
| Yusuf Öztürk, Nuray Yıldızoğlu-Arı, Ayça Özüarı and V. Melıh Altan | 1511 | The effects of glyburide and insulin on the decreased β-adrenergic responsiveness of the gastrointestinal tract in rats with non-insulin-dependent diabetes |
| Yusuf Öztürk, Nuray Yıldızoğlu-Arı, and V. Melıh Altan | 1519 | Altered α-adrenergic responses of vas deferens to noradrenaline and tyramine from rats with short- and long-term alloxan diabetes |


*Gen. Pharmac.* is Indexed/Abstracted in: Biosis Data., CAB Inter., Cam. Sci. Abstr., Chem. Abstr. Serv., Curr. Cont./Life Sci., CABS, Excerp. Med., Ind. Med., Curr. Cont. ISI/BIOMED Database, MEDLINE, PASCAL-CNRS Data., Res. Alert, Curr. Cont. Sci. Cit. Ind., Curr. Cont. SCISEARCH Data.

ISSN 0306-3623

GEPHDP 25(7) 1285–1526 (1994)

PERGAMON

0306-3623(1994)25:7;1-6

Pergamon

0306-3623(94)E0088-4

*Gen. Pharmac.* Vol. 25, No. 7, pp. 1465–1469, 1994
Copyright © 1994 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0306-3623/94 $7.00 + 0.00

# Effect of Aldonic Acids on the Uptake of Ascorbic Acid by 3T3 Mouse Fibroblasts and Human T Lymphoma Cells

MICHAEL J. FAY,[1] MARILYN J. BUSH[2] and ANTHONY J. VERLANGIERI[2]*

[1]*Department of Physiology, Dartmouth Medical School, 1 Medical Center Drive, Lebanon, NH 03756-0001 and* [2]*Department of Pharmacology, University of Mississippi, School of Pharmacy, University, MS 38677, U.S.A. [Fax. 601 232 5118]*

*(Received 4 February 1994)*

**Abstract**—1. Previously, we reported that calcium L-threonate caused a dose-related increase in uptake of ascorbic acid (AA) by human T-lymphoma cells. Preincubation of mouse fibroblasts with calcium L-threonate also resulted in a dose-related augmentation in uptake of AA as compared to non-treated controls.

2. Potassium L-lyxonate increased AA uptake by lymphoma cells, but did not significantly affect uptake by fibroblasts. Tartaric acid decreased uptake of AA by both cell lines.

3. Ouabain and dinitrophenol had no effect on AA uptake nor on the ability of threonate to augment AA uptake by fibroblasts. However, in T-lymphoma cells ouabain and dinitrophenol reduced AA uptake and prevented augmentation of AA uptake by calcium L-threonate.

## INTRODUCTION

The degradation of ascorbic acid (AA) involves a reversible oxidation to form dehydroascorbic acid. This is followed by an irreversible hydrolytic ring cleavage to form 2,3-diketo-L-gulonic acid. Diketo-L-gulonic acid may then undergo decarboxylation to form the aldonic acids, L-lyxonic acid and L-xylonic acid. An additional aldonic acid, L-threonic acid, results from the further oxidation of 2,3-diketo-L-gulonic acid. The *in vivo* formation of L-lyxonic and L-xylonic acids is documented in rat kidney homogenates (Kafnerr *et al.*, 1960; Kagawa *et al.*, 1960) and L-threonic acid in rat liver homogenates (Kagawa *et al.*, 1960). Experimental data concerning the production of these aldonic acids in humans is lacking.

A relationship between aldonic acids and AA was first reported by Thomas and Hughes (1983). The investigators found that scorbutic guinea-pigs treated with threonate had decreased AA content in the adrenals, testes, spleen and liver as compared to non-treated controls. These results led us to investigate the effects of acute treatment of human T-lymphoma cells with calcium L-threonate on AA uptake (Fay and Verlangieri, 1991). We found that when these cells were preincubated with threonic acid, AA uptake was increased in a dose-related fashion.

Additional evidence for a role of aldonic acids in AA bioavailability comes from animal experiments. Bush and Verlangieri (1987), dosed Sprague–Dawley albino rats with AA or Ester-C®, a calcium-ascorbate supplement containing 2% calcium-threonate, and monitored absorption rates and appearance of AA in the urine. The absorption rate of AA was more rapid and its appearance in the urine was later in the Ester-C®-treated group compared to the AA-treated group. Verlangieri *et al.* (1991) also examined the antiscorbutic effects of Ester-C® and AA in the ODS rat, which is unable to synthesize AA. The results of this study indicated a significant increase in body weight for the Ester-C®-treated group as compared to the AA-treated group. Also, the Ester-C®-treated group consistently scored lower than the AA-treated group using a scorbutic rating scale.

In the present studies, the effects of the aldonic acids, L-threonic acid and L-lyxonic acid, on AA uptake were further investigated using human T-lymphoma cells and mouse 3T3 fibroblasts.

*To whom all correspondence should be addressed.

## MATERIALS AND METHODS

### *Cell culture*

Human T-lymphoma cells (HUT 78, ATCC TIB 161, Gazdar *et al.*, 1980; Gootenberg *et al.*, 1981), and mouse 3T3 Swiss albino contact-inhibited fibroblasts (ATCC CCL 92) were obtained from the American Type Culture Collection (Rockville, MD). The lymphoma cells were maintained in RPMI-1640 medium (Sigma, St Louis, MO), and the fibroblasts in Dulbecco's modified Eagle's medium (Sigma), both supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY) and 50 $\mu$g/ml gentamicin (Sigma). The lymphoma cells were subcultured every 2–3 days at a density of $10^5$ cells/ml. Approximately every 3–4 days the fibroblasts were subcultured after exposure to 0.25% trypsin–EDTA (Gibco) and split at a high ratio (1:100) to maintain their characteristic contact inhibition.

### *Assay for cellular uptake*

Approximately 48 hr prior to experimentation, the T-lymphoma cells were subcultured at a density of $10^5$ cells/ml and the 3T3 fibroblasts were subcultured 1:5 into 6- or 12-well plates. Twenty hours prior to experimentation, cells were transferred to serum-free growth medium containing 0.1% bovine albumin (fraction V, Sigma). The cells were serum-starved to deplete serum proteins such as insulin, which has been shown to stimulate cellular AA uptake (Verlangieri and Sestito, 1981). For experimentation, confluent fibroblasts or $10^6$ viable T-lymphoma cells were washed 2 times with Ringer's balanced salt solution (pH 7.4). Cells were then preincubated for 1 hr at 37°C with 1.0 ml of Ringer's containing calcium L-threonate (0–30.5 mM, Pliva Chemical Co.), calcium L-lyxonate (30.5 mM, generously provided by Dr S. Rose, Arizona State University), calcium chloride (0–30.5 mM, Sigma), potassium chloride (30.5 mM, Sigma), L-tartaric acid (26.7 mM, Sigma), ouabain (0.1 mM, Sigma), or 2,4-dinitrophenol (0.1 mM, Sigma) as indicated in the results section. Following the preincubation, 1.0 ml of an AA solution was added, which contained 0.14 mM unlabelled AA (Sigma) and 0.125–0.250 $\mu$Ci of L-[1-$^{14}$C]AA (Dupont/NEN, specific activity 6.6 mCi/mmol). After 30 min, uptake was terminated by the rapid addition of 4.0 ml of ice cold Ringer's. Samples were washed 3 times with Ringer's to remove residual extracellular radioactivity. Cells were solubilized using 1.0 N NaOH. A 0.25 ml aliquot was neutralized with 0.25 ml of 1.0 N HCl and added to Instagel® liquid scintillation cocktail (Packard). Samples were dark-adapted overnight and counted 3 times for 5 min using a Packard model 3255 liquid scintillation counter. Counts per min (CPM) were converted to disintegrations per min (DPM) using a quench correction factor. Where indicated protein levels were determined using the method of Bradford (1976).

### *Statistical analysis*

Differences among groups were tested at $P \leq 0.05$ using either a one-way ANOVA followed by Duncan's multiple range test or Student's $t$-test, as indicated. The SPSS-X (SPSS Inc., Chicago) and SYSTAT (Systat Inc., Evanston, IL) statistical software programs were used for all of the analyses.

## RESULTS

The data for uptake are expressed as DPM/$10^6$ cells for the T-lymphoma cells, and as DPM/$\mu$g protein for the fibroblasts. Calcium L-threonate augmented AA uptake by the fibroblasts in a dose-related fashion, with the 23 and 30.5 mM doses being significant from the 0 mM control group (Fig. 1). The uptake ratio (experimental/control) of calcium L-threonate at 30.5 mM (1.7) was significantly greater than that of calcium chloride controls (1.1), but was insignificant at lesser concentrations. The potassium salt of L-lyxonic acid (30.5 mM) produced a slight increase (23%) in ascorbic uptake in fibroblasts [Fig. 2(A)], but significantly increased AA 198% above the control group in T-lymphoma cells [Fig. 2(B)]. This effect of potassium L-lyxonate on the lymphoma cells was not due to potassium ion, as the uptake ratio of potassium L-lyxonate (3.0) was signifi-

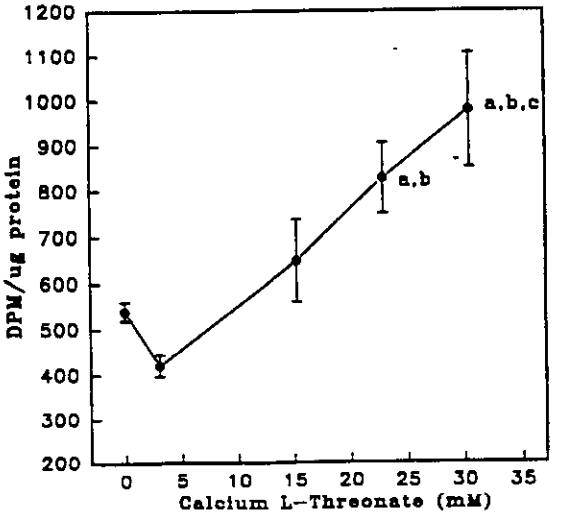



Fig. 1. Effect of 1 hr preincubation of 3T3 mouse fibroblasts with calcium L-threonate (0–30.5 mM) on the uptake (30 min) of AA (0.07 mM unlabelled AA/0.250 $\mu$Ci L-[1-$^{14}$C]AA). Uptake is expressed as DPM/$\mu$g protein ± SEM, $n = 6$ per group, [a] significantly different from 0 mM, [b] significantly different from 3 mM, [c] significantly different from 15 mM (ANOVA followed by Duncan's MRT, $P \leq 0.05$).

cantly greater than that of potassium chloride (0.8). The effect of tartaric acid at 26.7 mM was similar in both cell lines. In mouse fibroblasts, DPM/$\mu$g protein was 321 in control cultures and 126 in treated cultures, while in lymphoma cells DPM/$10^6$ cells were 9466 and 5483, respectively. These results indicate a significant decrease in AA uptake when tartaric acid is present in the medium. Ouabain (0.1 mM) and 2,4-dinitrophenol (0.1 mM) significantly decreased AA uptake by the T-lymphoma cells and prevented calcium L-threonate from augmenting AA uptake [Fig. 3(B)]. However, in the fibroblasts ouabain (0.1 mM) and 2,4-dinitrophenol (0.1 mM) did not significantly affect AA uptake and did not appear to inhibit the ability of calcium L-threonate (30.5 mM) to increase AA uptake [Fig. 3(A)].

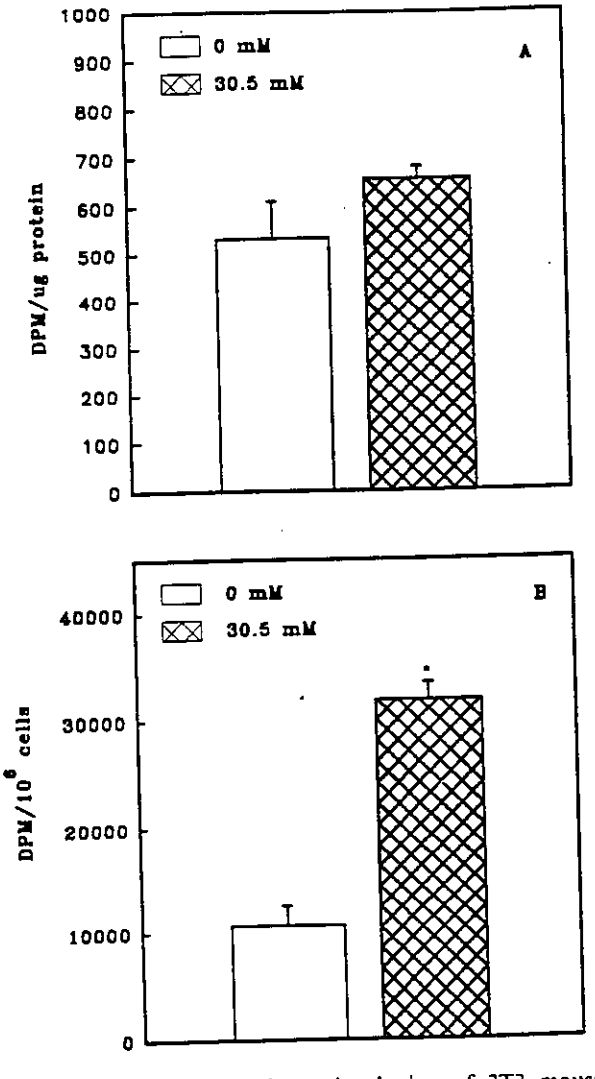



Fig. 2. (A) Effect of 1 hr preincubation of 3T3 mouse fibroblasts with potassium L-lyxonate (30.5 mM) on the uptake (30 min) of AA (0.07 mM unlabelled AA/0.250 $\mu$Ci L-[l-$^{14}$C]AA. Uptake is expressed as the mean DPM/$\mu$g protein $\pm$ SEM, $n = 4$ per group. (B) Effect of 1 hr preincubation of human T-lymphoma cells with potassium L-lyxonate (30.5 mM) on the uptake (30 min) of AA (0.07 mM unlabelled AA/0.125 $\mu$Ci L-[1-$^{14}$C]AA). Uptake is expressed as DPM/$10^6$ cells $\pm$ SEM, $n = 3$ for the 0 mM group, $n = 4$ for the 30.5 mM group, * significantly different from control (Student's $t$-test, $P \leq 0.05$).

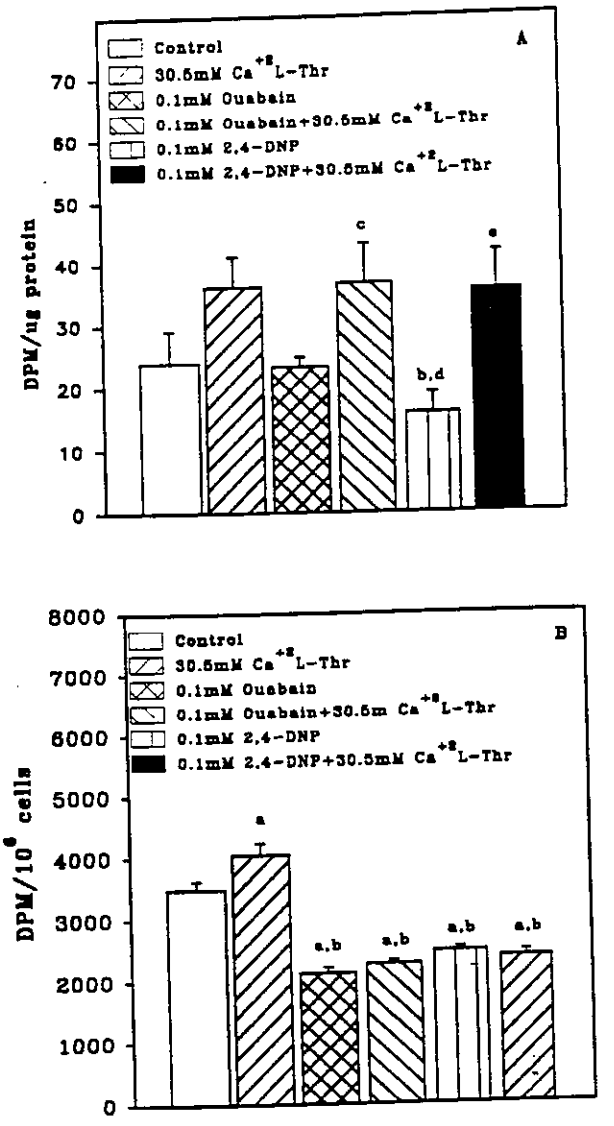



Fig. 3. (A) Effect of ouabain (0.1 mM) and 2,4-dinitrophenol (0.1 mM) preincubation (1 hr) on the ability of calcium L-threonate (30.5 mM) to increase the uptake (30 min) of AA (0.07 mM unlabelled AA/0.250 $\mu$Ci L-[1-$^{14}$C]AA by 3T3 mouse fibroblasts. Uptake is expressed as mean DPM/$\mu$g protein $\pm$ SEM, $n = 5$ for the control group, $n = 6$ for all other groups, [b]significantly different from the 30.5 mM calcium L-threonate group, [c]significantly different from the 0.1 mM 2,4-dinitrophenol group, [d]significantly different from the 0.1 mM ouabain + 30.5 mM calcium L-threonate group, [e]significantly different from the 0.1 mM 2,4-dinitrophenol group (ANOVA followed by Duncan's MRT). (B) Effect of ouabain (0.1 mM) and 2,4-dinitrophenol (0.1 mM) preincubation (1 hr) on the ability of calcium L-threonate (30.5 mM) to increase the uptake (30 min) of AA (0.07 mM unlabelled AA/0.125 $\mu$Ci L-[1-$^{14}$C]AA) by human T-lymphoma cells. Uptake is expressed as mean DPM/$10^6$ cells $\pm$ SEM, $n = 3$ per group, [a] significantly different from the 0 mM group, [b] significantly different from the 30.5 mM calcium L-threonate group (ANOVA followed by Duncan's MRT, $P \leq 0.05$).

## DISCUSSION

Few studies have focused on the biological actions of aldonic acid metabolites resulting from the oxidative metabolism/degradation of AA. Thomas and Hughes (1983) were the first to report an interaction between threonic acid and AA. They found decreased AA content in certain scorbutic guinea-pig organs after 4- and 28-day treatments with threonic acid. The same authors also demonstrated an apparent lack of toxicity of dietary calcium threonate administered to rats and mice in a chronic study (Thomas and Hughes, 1985). Previously we reported that acute treatment of human T-lymphoma cells with calcium L-threonate augmented the uptake of physiological levels of AA (Fay and Verlangieri, 1991). In the present studies, we extend these findings to mouse 3T3 fibroblasts. At 30.5 mM the effect of calcium L-threonate on AA uptake was significantly greater than that of the calcium chloride control.

Taken together with the reports of Thomas and Hughes, the collective data suggest that threonic acid affects the availability of AA both *in vivo* and *in vitro*. In agreement with Thomas and Hughes' *in vivo* toxicity study (1985), we have demonstrated *in vitro* that calcium L-threonate is non-toxic to both cell lines (unpublished observation), as measured by trypan-blue exclusion.

The effect of potassium L-lyxonate on AA uptake by the T-lymphoma cells and the 3T3 mouse fibroblasts was examined to determine if other aldonic acids resulting from AA metabolism augment cellular AA accumulation. A dose of 30.5 mM potassium L-lyxonate significantly increased AA uptake by the T-lymphoma cells. Although AA uptake was increased, the effect of potassium L-lyxonate on mouse fibroblasts was not significant.

Further studies revealed that the four-carbon aldonic-like acid, L-tartaric acid, significantly decreased AA uptake by the T-lymphoma and 3T3 cells. A dose of 26 mM L-tartaric acid was used to approximate the amount of threonate present in 30.5 mM calcium L-threonate. These results indicate some specificity on the part of threonate and lyxonate. This apparent antagonistic effect of L-tartaric acid was not due to cellular toxicity as measured by the accumulation of trypan-blue (unpublished observation).

To investigate whether the effect of threonic acid was dependent on sodium, we included ouabain, an inhibitor of $Na^+/K^+$ ATPase, in the incubation medium. To determine if the effect of threonate was energy-dependent, we added 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, to the incubation medium. Results of these studies indicated that AA uptake by the human T-lymphoma cells was sodium and energy dependent and that in the presence of ouabain and 2,4-dinitrophenol calcium L-threonate was unable to augment AA accumulation. However, in the fibroblasts, the uptake of AA did not appear to be sodium or energy dependent and calcium L-threonate was effective at augmenting AA uptake. Collectively, these studies suggest that the effect of calcium L-threonate is acting at the level of an AA transporter. Differences in the magnitude of the calcium L-threonate effect in the T-lymphoma cells and 3T3 fibroblasts may be attributable to variations in cultured cells over time and variations among different batches of threonate.

In the present studies, the investigators made no attempt to distinguish between AA and dehydroascorbic acid. However, data indicate that the half-life of AA in culture medium without added glutathione is 3.7 hr (Schorah, 1992) and the incubations we used (30 min) were well below this half-life.

The results reported here indicate that aldonic acids resulting from AA metabolism/degradation may have a modulatory effect on AA transport. In addition to its role in the prevention of scurvy, AA has been implicated in immune cell function (Anderson, 1981; Prinz *et al.*, 1977), cholesterol metabolism (Ginter, 1973), the prevention of cardiovascular disease (Verlangieri *et al.*, 1985), and also in cancer treatment and/or prevention (Cameron and Pauling, 1976; Wittes, 1985). More recently, AA has been shown to suppress the replication of the human immunodeficiency virus (HIV) in infected human T-lymphoma cells (Harakeh *et al.*, 1990). A significant augmentation of delivery of AA to cells may have important clinical impact on the treatment or prevention of several diseases.

*Acknowledgements*—The authors are grateful to the InterCal Corp. (Prescott, AZ) for their support of this work, as well as to Dr Seth Rose (Arizona State University, Tempe, AZ) for providing calcium L-threonate. We also appreciate the clerical assistance of Ms Pat Montgomery.

## REFERENCES

Anderson R. (1981) Ascorbic acid and immune function. In *Vitamin C: Ascorbic Acid* (Edited by Counsell I. N. and D. H. Hornig), pp. 249–272. Applied Science Publishers, London.

Bush M. J. and Verlangieri A. J. (1987) An acute study on the relative gastrointestinal absorption of a novel form of calcium ascorbate. *Res. Commun Chem. Path. Pharmac.* **57**, 137–140.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.

Cameron E. and Pauling L. (1976) Supplemental ascorbate in the supportive treatment of cancer: prolongation of

survival times in terminal human cancer. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3685–3689.

Fay M. J. and Verlangieri A. J. (1991) Stimulatory action of calcium L-threonate on ascorbic acid uptake by a human T-lymphoma cell line. *Life Sci.* **49**, 1377–1381.

Gazdar A. F., Carney D. N., Bunn P. A., Russell E. F., Jaffe E. S., Shector G. P. and Guccion J. C. (1980) Mitogen requirements for *in vitro* propagation of cutaneous T-cell lymphomas. *Blood* **55**, 409–417.

Ginter E. (1973) Cholesterol: Vitamin C controls, its transformation to bile acids. *Science* **179**, 702–704.

Gootenberg J. E., Ruscetti F. W., Mier J. W., Gazdar A. and Gallo R. C. (1981) Human T-lymphoma and leukemia cell lines product and respond to T-cell growth factor. *J. Exp. Med.* **154**, 1403–1418.

Harakeh S., Jariwalla R. J. and Pauling L. (1990) Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely affected cells. *Proc. natn. Acad. Sci. U.S.A.* **87**, 7245–7249.

Kafner K., Ashwell G. and Burns J. J. (1960) Formation of L-lyxonic and L-xylonic acids from L-ascorbic acid in rat kidney. *J. biol. Chem.* **235**, 2518–2521.

Kagawa Y., Mano Y. and Shimazono N. (1960) Biodegradation of dehydro-L-ascorbic acid; 2,3-diketo-aldonic acid decarboxylase from rat liver. *Biochim. biophys. Acta* **43**, 348–349.

Prinz W., Bortz R., Bregin B. and Hersch M. (1977) The effect of ascorbic acid supplementation on some parameters of the human immunological defense system. *Int. J. Vit. Nutr. Res.* **47**, 248–257.

Schorah C. J. (1992) The transport of vitamin C and effects of disease. *Proc. Nutr. Soc.* **51**, 189–198.

Thomas M. and Hughes R. E. (1983) A relationship between ascorbic acid and threonic acid in guinea-pigs. *Food Chem. Toxic.* **21**, 449–452.

Thomas M. and Hughes R. E. (1985) Evaluation of threonic acid toxicity in small animals. *Food Chem.* **17**, 79–83.

Verlangieri A. J. and Sestito J. (1981) Effect of insulin on ascorbic acid uptake by heart endothelial cells: Possible relationship to retinal atherogenesis. *Life Sci.* **29**, 5–9.

Verlangieri A. J., Kapeghian J. C., El-Dean S. and Bush M. J. (1985) Fruit and vegetable consumption and cardiovascular mortality. *Med. Hypoth.* **16**, 7–15.

Verlangieri A. J., Fay M. J. and Bannon A. W. (1991) Comparison of the anti-scorbutic activity of L-ascorbic acid and Ester-C in the non-ascorbate synthesizing osteogenic disorder (ODS) rat. *Life Sci.* **48**, 2275–2281.

Wittes R. E. (1985) Vitamin C and cancer. *New Engl. J. Med.* **312**, 178–179.

# COMPARATIVE EFFICACY OF

# INTER-CAL

# "ESTER-C" CALCIUM ASCORBATE

Prepared for

Inter-Cal Corporation
533 Madison Avenue
Prescott, Arizona 86301

Prepared by

Howard Hunt, Ph.D.
Thomas Rice, Ph.D.

The Life Management Group, Inc.
3262 Holiday Court
Suite 204
La Jolla, California 92037
(619) 452-5554

March 1995

## SUMMARY

The cellular utilization of vitamin C in Inter-Cal Ester-C calcium ascorbate, with and without standardized 3% calcium threonate, was compared to pure ascorbic acid by measuring changes in white blood cells following a single oral dose of 1000 mg of vitamin C (as ascorbic acid contained in product) in subject groups composed of 17-18 healthy men.

Subjects acclimatized to a low-vitamin C diet for 12 days prior to supplementation. Blood samples were taken at 0 hr and again at 1, 2, 4, and 24 hr following ingestion of the test dose. Measurements of ascorbate concentration in white blood cells (buffy coat) were made by specific HPLC methods.

White cell ascorbate in the pure ascorbic acid group gradually declined by 24 hr but remained high in both the Ester-C and the Ester-C standardized with 3% threonate groups. At 24 hr, both Ester-C groups showed white cell levels about twice as great as those in the pure ascorbic acid group. The Ester-C product containing standardized threonate produced higher white cell ascorbate levels than the native Ester-C product. Ascorbate levels at 24 hr in white cells of the Ester-C calcium ascorbate group were significantly greater than those of the pure ascorbic acid group; this difference was highly statistically significant ($P = 0.0072$).

*This study found a highly significant enhancement of cellular uptake of ascorbate from Ester-C calcium ascorbate compared to pure ascorbic acid, as judged by white blood cell ascorbate levels.*


## BACKGROUND AND INTRODUCTION

The Inter-Cal Corporation of Prescott, Arizona, manufactures a number of vitamin C products by a patented process that produces a neutral ascorbate containing significant concentrations of vitamin C "metabolites", or biochemical intermediates, such as dehydroascorbate, diketogulonate, threonate, xylonate, and lyxonate. Their leading product, called "Ester-C calcium ascorbate", is a hemi-calcium salt of ascorbic acid containing up to 6% dehydroascorbate and about 1% threonate generated by the manufacturing process. Inter-Cal maintains that the Ester-C ascorbates are more bioavailable than ordinary ascorbic acid because one or more of the metabolites enhances the intestinal absorption and cellular utilization of oral ascorbate supplements.

Considerable published and unpublished research on the biological assimilation of Ester-C ascorbate has been carried out, most notably that of Dr. Anthony Verlangieri of the Department of Pharmacology at the University of Mississippi and of Dr. Seth Rose of the Department of Chemistry at Arizona State University. Dr. Rose's work was instrumental in identifying the nature and amount of the "metabolites". Dr. Verlangieri and his colleagues have shown superior bioavailability of the Ester-C calcium ascorbate in animal models (Bush and Verlangieri, 1987; Verlangieri, Fay, and Bannon, 1991). They have recently demonstrated that small amounts of threonate stimulate the transport of ascorbate across cell membranes in vitro (Fay, Bush, and Verlangieri, 1994). Some preliminary human efficacy studies were performed several years ago in support of one or more of Inter-Cal's patent applications regarding the manufacturing process for Ester-C ascorbates. These studies showed superior bioavailability of the Ester-C ascorbate compared to ascorbic acid, as measured by blood levels and urinary excretion following single oral doses of ascorbate products (Wright and Suen, 1990).

Many consumers and health professionals believe that ascorbate has both nutritional and therapeutic properties when administered at high dosages. The high dosages required to achieve these effects are thought to permit accumulation of critical concentrations of ascorbate within cells and tissues. Bioavailability of ascorbate, then, may be reflected most meaningfully by cellular concentration of the vitamin.

Investigators often make use of plasma (or serum) ascorbate concentrations following oral doses to compare relative bioavailability, on the assumption that more efficient intestinal absorption leads to higher plasma levels. The concentration of ascorbate that remains in the circulation (i.e., in the plasma fraction) appears, however, to be maintained homeostatically within very narrow limits to promote optimum tissue function. Plasma ascorbate levels, then,

may not reflect true bioavailability but may merely represent ascorbate in transit or in equilibrium with tissues (Bergsten, Moura, Atwater, and Levine, 1994).

Urine ascorbate concentrations have been used similarly to draw conclusions about relative bioavailability. Previous work has suggested that less ascorbate appeared in the urine during the 24 hours following an oral dose of Ester-C ascorbate than of pure ascorbic acid. Investigators have concluded that lower urinary excretion reflects higher tissue retention and utilization of the Ester-C product.

Inter-Cal has an interest in documenting the efficacy of its products to maintain assurance of quality and also to document claims of superior bioavailability. The proposed human trial will compare the bioavailability of ascorbate in Ester-C calcium ascorbate against other product forms, using biochemical measurements on white blood cells. This biological material is readily available at low risk to human subjects. In addition, this study may provide further documentation of the role of threonate in facilitating the absorption and utilization of ascorbate in the human.

The objectives of this study were to determine if the vitamin C contained in Inter-Cal Ester C calcium ascorbate is more utilizable than other product form(s) of ascorbic acid and to determine if additional calcium threonate enhances the utilization of ascorbate contained in Ester-C calcium ascorbate.

We report the results of a study of Ester-C's effectiveness in promoting cellular uptake of vitamin C in white blood cells, which are a key component of immune function and resistance to infection. In the present efficacy study, volunteers ingested a single oral dose of 1000 mg of vitamin C in various product forms: (a) Inter-Cal Ester-C calcium ascorbate; (b) Inter-Cal Ester-C calcium ascorbate plus calcium threonate standardized to 3%; and (c) pure ascorbic acid. Cellular utilization and antioxidant potential was assessed by ascorbate concentration of white blood cells. This study was part of a larger coordinated nutrition study in which many samples of biological fluids were collected for other purposes. Data for ascorbate concentration of the blood plasma in these subjects, for example, are not available, since this fraction was reserved to meet other study requirements. Additional data may be reported later in a separate publication.

## METHODS AND MATERIALS

### Subject Population, Recruitment, and Selection

Population and recruitment. Healthy adult men, aged 21-50, were recruited among participants in corporate aerobic fitness programs administered by the Life Management Group throughout San Diego County. In order to be eligible for participation in the study and subsequent orientation sessions, potential subjects were required to meet the following criteria: (a) good health with no evidence of cardiovascular disease; (b) not diabetic; (c) willingness and ability to withstand repeated venipuncture without nausea or fainting; (d) willingness and ability to consume large doses of vitamin supplements without nausea or intestinal disturbance; (e) willingness and ability to consume a diet low in Vitamin C (mainly excluding fruit, vegetables, juices, and vitamin supplements) for one week. Only male subjects were recruited in order to reduce intersubject variability due to gender and because of evidence that females have a higher Vitamin C requirement than males. Potential subjects understood they would receive an honorarium for successful compliance with the research protocol.

Number of subjects. The study design called for 3 groups in order to compare 3 test products. Preliminary work suggested that a group size of 18 would be required to compensate for potential study dropouts or missing data and to test statistical hypotheses about product differences, given the inherent intersubject variability and variability in biochemical assays for ascorbic acid in biological fluids. Thus, 54 subjects (3 groups x 18 subjects each) were selected from the candidate pool.

Randomization and selection. Groups were matched as closely as possible by age, weight, body mass index ("weight for height"), use of tobacco, use of vitamin supplements, and committment to regular exercise. The last three variables were coded dichotomously (0=no, 1=yes). Matching was performed by a computer program, based on "bootstrap" statistical methods, that randomly assigned 54 individuals

in the candidate pool into 3 groups of 18 members each and recorded the coefficient of variation (relative standard deviation) between the 3 resulting groups with respect to the 6 variables above. Because of the very large number of potential combinations of candidate groupings, randomized assignment was carried out only 200,000 times, and final assignment was determined by the groupings that yielded the smallest sum of coefficients of variation of the 6 given variables.

## Subject Protection

Informed consent. Informed consent was given in writing by the subjects following verbal and written disclosure of risk and benefit. The consent form reviewed study objectives, discussed risks and benefits of participation, and stressed that full compliance with all aspects of the study protocol and blood draws was required as a condition of receiving payment for participation. The same information was given orally to each subject. A signed and witnessed consent form was obtained from each subject.

Institutional review. The protocol and experimental design of this study was reviewed by the 6-member Institutional Review Board of the Life Management Group, following standards and procedures similar to those used by the University of California San Diego Human Subjects Protection Committee.

Privacy and anonymity. Subject names were used for handouts and checklists for use of administrative staff prior to and during dosage and blood drawing. Each subject was assigned a number to code all labels for laboratory use. No names were used on any materials containing analytical or statistical results.

## Description of Experiment

Vitamin C washout and dietary restriction. In order to reduce vitamin C stores in the body and to enhance efficiency of absorption, participants consumed no vitamin C supplements and received verbal and written counseling about how to reduce dietary vitamin C intake during a 12-day acclimatization ("washout") period. Counseling stressed the need (a) to avoid vitamin C supplements; (b) to avoid eating fruits, vegetables, and juices from these sources; (c) to avoid fortified cereals; (d) to avoid foods containing fruits and vegetables as ingredients; (e) to avoid beverages containing fruit extracts or reconstituted juices; (f) to read food labels for nutritional information; and (g) to read food labels for ingredients that might be restricted.

Sequestration of subjects. Approximately 12 hours prior to vitamin C dosing, selected subjects checked into a local hotel for a 2-night stay. All subjects ate dinner before 8:00 PM of the night preceding the vitamin C dosing and blood sampling; they were allowed no food, but unlimited water, thereafter. This meal, as well as the meal served on the following evening, contained a minimum of vitamin C and was planned by the hotel chef in consultation with a nutritionist from the Life Management Group. Subjects were asked to contain their activities for the next 1-2 days to the areas around the hotel and the site of the experiment (within walking distance of the hotel). Subjects were allowed unlimited access to water but ate only low Vitamin C food provided by the Life Management Group for the duration of the experiment. Meals and hotel accommodations were provided by the Life Management Group at no expense to the subjects.

Vitamin C dosage. After overnight fasting, subjects ingested a single fixed dose of 1000 mg of ascorbic acid as product dissolved in 250 mL of reconstituted apple juice containing no significant vitamin C. Subjects had been counseled to avoid taking anti-inflammatory or analgesic drugs that might slow blood clotting time and promote bruising at the site of venipuncture. After one hour, subjects were allowed unlimited access to water and controlled access to low-vitamin C foods for the next 24 hours, until the last blood sample had been collected the following day.

Blood collection. Blood was collected into 5-mL "Vacutainer" tubes, containing lithium heparin anticoagulant, at 0 hours and at target times of 1, 2, 4, and 24 hr following dosage. Tubes were labeled with a randomly assigned number; this number, along with the subject's number and the time of blood collection, was recorded in a separate location for later decoding. Whole anticoagulated blood was kept on ice and the white cell fraction was separated without delay (see below).

Payment of subject honorarium. Following the 24-hr blood draw, each subject signed for and received cash payment of the agreed-upon honorarium.

## Study Double Blinding

Administrative staff of Life Management Group coded and distributed products in such a way that the investigators, analysts, and participants were blinded to the association between product and subject. The code was revealed at the conclusion of the data collection phase, in order to permit statistical analysis of grouped data.

## Study Protocol (Outline)

| | |
|---|---|
| - 12 dy | Dietary vitamin C restriction |
| - 12 hr | Sequester subjects; Start fasting |
| 0 hr | Blood sample (pre-treatment); Vitamin C oral supplementation (1000 mg per subject) |
| + 1 hr | Blood sample (post-treatment) |
| + 2 hr | Blood sample (post-treatment) |
| + 4 hr | Blood sample (post-treatment); Sequester subjects |
| + 24 hr | Blood sample (post-treatment); Dismiss subjects |

## Source of Materials

Test products. Pure ascorbic acid was obtained as the U.S.P. crystalline material. Ester-C calcium ascorbate was obtained from Inter-Cal. The Ester-C standardized to 3% threonate was prepared by dry mixing the same lot of Ester-C calcium ascorbate with sufficient calcium threonate to provide a total of 3% by weight of threonate (as the L-threonic acid hemicalcium salt) in the final mixture.

Laboratory chemicals. All chemical reagents were of the highest commercially available purity. Water was purified by deionization, reverse osmosis, and sub-boiling distillation; this water was also used for preparation of chromatographic solvents.

## Assay of Ascorbate and Threonate in Test Products

Ascorbic acid content of the products used in this study was determined as reducing equivalents by iodometric titration, as described in USP XXIII (p. 130). Threonate concentration was determined by high performance liquid chromatography (HPLC) methods. Analyses were performed by Alan Wiseman, Manager of Quality Control of the Inter-Cal Corporation. Documentation of these analytical methods and results is contained in his internal memorandum of 17 November 1994. Determination of dehydroascorbate levels in the test products was not a study requirement.

Table 1 lists ascorbate and final threonate content of each test product. Ascorbate content of the pure ascorbic acid, the Ester-C calcium ascorbate, and the Ester-C calcium ascorbate standardized with 3% threonate was calculated on the basis of purity found by iodometric titration.

## Processing of Blood Fractions

White blood cells. Whole blood, collected in lithium heparin anticoagulant, was placed on ice immediately. Blood was centrifuged in a swinging-bucket rotor at 1,000 x g for 10 min. Plasma was pipetted off with care so as not to disturb or remove the buffy coat layer. White blood cells were isolated from the buffy layer by density-gradient centrifugation by an unpublished adaptation of the method described by Evans, Currie, and Campbell (1982) [privileged communication from Drs. Joe Voland and Dan Gelvan of the Department of Biology, University of California San Diego]: The buffy coat (white cells) together with some red cells were gently stirred and pipetted off into a clean test tube. The cells (measured volume=1 volume) were diluted with 3 volumes of physiological saline; this cell suspension was then pipetted gently on top of 2 volumes of "Ficoll-Paque" (Ficoll 400/sodium diatrizoate; density 1.077 g/mL; Pharmacia Biotech) in a 15-mL clear conical polystyrene centrifuge tube. Tubes were centrifuged in a cold swinging-bucket rotor at 2,000 x g for 20 min, after which white cells banded in the Ficoll-saline interphase and red cells layered on the bottom of the Ficoll layer. White cells were carefully pipetted into another 15-mL conical tube, 3 mL

of saline were added to dilute the Ficoll medium, and the suspension was centrifuged in the cold at 2,000 x g for 5 min. The supernatant was removed, the cells at the bottom of the tube were resuspended in saline, and centrifugation was repeated to sediment the white cells. The cells were resuspended in 1.0 mL of saline, and 0.1-mL aliquot of cell suspension was withdrawn for cell counting (see below). The remaining cells were centrifuged again, the saline supernatant was drained briefly, and the cells were finally suspended in 0.9 mL of ice-cold 1.0 mM EDTA in 90% methanol for immediate storage at -70oC in polycarbonate tubes.

White cell counting. Cell suspension (0.1 mL) was added to 15 mL of isotonic diluent ("Hematall", Fisher Diagnostic). Cell counting was performed in triplicate on 0.5-mL aspirations in a Coulter Cell Counter (Model 2b, 100-um aperture). Raw counts were corrected for dilution, aspiration volume, and counting efficiency according to a nomogram contained in the operating manual.

Stability of ascorbate. We confirmed that white cell samples preserved in this way were stable for several months. Properly preserved, samples showed little or no dehydroascorbic acid (Dhariwal, Hartzell, and Levine, 1991).

## Assay of Ascorbate in Blood Fractions

HPLC system. A Rainin Instruments "Rabbit" HPLC system with a Kauer ultraviolet (UV) detector was used with automated computer control and data acquisition. C-18 columns (5-um bead; 4.6 mm diameter) of varying manufacture and length were employed as the stationary phase according to the sample load; all were used with a 3-cm Rainin C-18 guard column. Mobile phase was 5% (v/v) acetonitrile, 1% (v/v) acetic acid, 1.8 mM tetrabutylammonium hydroxide, adjusted to pH 4.8 with 1.0N NaOH. Ascorbate was quantitated by peak area of effluent absorbance at 265 nm; dehydroascorbate has no appreciable UV absorbance at this wavelength. Multipoint standardization was performed on a frequent schedule over a range of 100-1000 pmoles of ascorbic acid dissolved in the EDTA/methanol solvent and stored along with blood fractions at -70oC. Effluent absorbance data were corrected for baseline shifts and anomalies due to injection of the EDTA/methanol solvent in which samples were stored. Ascorbic acid content of injected samples was calculated from a linear regression equation obtained with the standards and then corrected for dilution with the EDTA/methanol solvent used to preserve the samples in the frozen state.

White blood cells. Prior to HPLC analysis, samples of white blood cells that had been frozen in EDTA/methanol were thawed, sonicated for 20 sec on ice at high power, and centrifuged to remove cell debris. Ascorbic acid was typically determined in duplicate by injection of a 20-to-200-uL aliquot, depending on cell recovery following density-gradient cell isolation. A 3-cm Perkin-Elmer C-18 column was used at a flow rate of 1.0 mL/min, and data were acquired for 1.5 min. Because efficiency of cell recovery was not controllable, ascorbic acid measurements could not be related to concentration in whole blood. Rather, ascorbic acid content of samples determined by HPLC was normalized as mass per cell.

## Statistical Tests and Mathematical Computations

Comparison of arithmetic means of grouped data was carried out by a 2-sample t test for unpaired (independent) data, which allows comparison of groups with unequal variance and different sample sizes that arise unavoidably by the exclusion, unavailability, or loss of sample data. Values of t were calculated by the formula given by Rice (1988, p. 349), using an estimate of pooled sample variance, in which the contribution of each variance is weighted in proportion to its sample size. The sample standard deviation, s, used in these calculations was computed by the usual "n-1" formula (see Dixon and Massay, 1969; p. 28), which is implemented in Borland Quattro as the @STDS spreadsheet function. Probabilities (P) based on the statistic were computed as the tail area of the t distribution, with adjusted degrees of freedom calculated by the formula of Rice (1988, p. 356). Calculation of areas of the t distribution used an approximation of a Chebyshev polynomial (Gentleman and Jenkins, 1968).

A 5% level of significance (i.e., chance of error) was chosen to evaluate the statistical tests. Statements about whether a particular single value or mean was greater or less than a reference mean were evaluated by one-tailed tests and were considered to be supported if $P < 0.05$. Statements about whether a particular single value or mean was different from a reference mean were evaluated by two-tailed tests and were considered to be supported if $2P < 0.05$. A statistical test of the latter hypothesis is equivalent to answering the question, "Could a difference of this absolute magnitude between two average values be due to (a 1 in 20) chance alone?"

Calculations were performed on a personal computer with a 80486 microprocessor/numeric coprocessor, running MS-DOS Version 6.22 and the 4DOS Version 5.5 command interpreter as a "virtual DOS machine" under the OS/2 Version 3.0 operating system. Statistical algorithms were programmed in Pascal using the Stony Brook Version 6.14e optimizing compiler to implement extended-precision floating-point arithmetic.

## RESULTS AND DISCUSSION

### Subject Matching

54 subjects were selected to begin this study. One subject assigned to the ascorbic acid group failed to complete study requirements and was eliminated from the pool of data. Vital statistics and health habits of the remaining subjects were compared by group. No significant differences between groups with respect to the 6 primary subject variables (age, weight, body mass index, use of tobacco, use of vitamin supplements, and committment to regular exercise) were found (see Tables 2A and 2B), so elimination of the one subject did not significantly perturb the group equalization produced during the initial randomization.

### Loss and Elimination of Data

Some subject data were not available. Certain subjects did not tolerate phlebotomy particularly well, so some blood samples either could not be collected in sufficient volume for processing or were hemolyzed so extensively that white blood cells could not be fractionated. Some white blood cell fractionations gave no recovery because the densities of the cells and of the gradient medium were mismatched. Unavoidably, some samples were lost in the laboratory during processing or in storage due to mishandling, mislabeling, breakage, or equipment failure.

Some data were discarded objectively as outliers. All true zero values ("none detected" after confirmation by repeat analysis) were discarded, because HPLC methodology is sensitive enough always to yield a non-zero value and because none of the subjects was scorbutic. Picking outliers was problematic because the statistical distribution of data values tended to be skewed, i.e., non-normal. Values that remained outside the 99.5% confidence interval after repeat analysis were always suspicious and were almost always discarded.

The net result of the data loss was a difference in sample size as well as sample variance at each timepoint. Accordingly, the usual paired (i.e., "before and after") statistical comparisons could not be made. Instead, statistical comparisons of unpaired grouped data was conducted with tests that account for unequal variances.

A large relative standard deviation at many timepoints is evident in the data Tables. Error bars have not been added to data in the Figure, because their breadth obscured the datapoints and the connecting lines.

### Timing of Blood Collection

Target times of 1, 2, 4, and 24 hr following dosage with test products were chosen for blood sampling. On average, the actual elapsed time following dosage was somewhat less than the target time (see Table 3). In spite of this, the average interval was approximately the same for each of the 3 groups, and no statistically significant differences were observed between corresponding timepoints. Therefore, throughout the rest of this discussion, we will refer exclusively to the target times as if they were

identical for corresponding timepoints in each group.

## White Blood Cell Ascorbate

The Figure shows the timecourse of white blood cell (buffy coat) ascorbate following ingestion of a single oral dose of ascorbic acid. Average ascorbate levels shifted abruptly during the first 4 hrs of blood sampling. We noted a fall, rise, and fall in ascorbate in all groups. The fall within the first hour might be due to the stress of the phlebotomy. Further changes most likely reflect uptake and equilibration of circulating ascorbate into the white blood cells. These changes did not reach the level of statistical significance. White cell ascorbate in the pure ascorbic acid group, however, gradually declined by 24 hr but remained high in both the Ester-C and the Ester-C standardized with 3% threonate groups. At 24 hr, both Ester-C groups showed white cell levels about twice as great as those in the pure ascorbic acid group (see Figure). The Ester-C product containing standardized threonate produced higher white cell ascorbate levels than the native Ester-C product. Ascorbate levels at 24 hr in white cells of the Ester-C calcium ascorbate group were significantly greater than those of the pure ascorbic acid group (Table 4); this difference was highly statistically significant ($P = 0.0072$). The corresponding difference between the Ester-C standardized threonate group and the pure ascorbic acid group did not reach the level of statistical significance because of a high standard deviation in the former group (Table 4).

Analysis of white blood cell data by individual was not successful. Because of missing data at certain timepoints, it was not feasible to analyze these data by percentage change. Data treatment as a percentage difference did not reduce, but rather greatly increased, the sample variances. We were thus not able successfully to integrate individual white blood cell responses by summing individual changes over "baseline", because a stable baseline could not be determined analytically. Intrasubject variability in HPLC assay prevents us from obtaining a single, stable value -- say, for a baseline, pre-supplementation concentration -- that can then be subtracted from post-supplementation data. This is particularly true for white blood cell ascorbate measurements, since the uncertainty of the blood cell count is added to the uncertainty of the HPLC value.

## CONCLUSIONS

This study found a highly significant enhancement of cellular uptake of ascorbate from Ester-C calcium ascorbate compared to pure ascorbic acid, as judged by white blood cell ascorbate levels.

## ACKNOWLEDGEMENTS

Bruce Van Dyke (B.S., 1980, University of California San Diego), Staff Research Associate in the Biology Department of the University of California San Diego, headed the blood fractionation team, developed the HPLC methods, and supervised all technical aspects of this study. Graduate and undergraduate students and staff laboratory technicians in the Biology Department provided the remainder of the technical assistance. We particularly thank Elizabeth Machunis, Diana Yeh, Ian McLoughlin, John Esser, and Donita Sanders for their help and dedication. Dorinda ("Dee") Boyd headed the team of phlebotomists from the Nichols Institute in San Diego.

The Inter-Cal Corporation of Prescott, Arizona, provided all financial support for this investigation.

## REFERENCES


Dhariwal, K. R., Hartzell, W. O., and Levine, M. (1991) Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. Am. J. Clin. Nutr. 54: 712-716.

Dixon, W. J., and F. J. Massey (1969) Introduction to Statistical Analysis (3rd ed.). New York: McGraw-Hill.

Evans, R. M., L. Currie, and A. Campbell. (1982) The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. Brit. J. Nutr. 47: 473-482.

Gentleman, W. M., and M. A. Jenkins. (1968) An approximation for Student's t-distribution. Biometrika 55: 571-572.

Rice, J. A. (1988) Mathematical Statistics and Data Analysis. Pacific Grove, CA: Wadsworth & Brooks/Cole Advanced Books & Software.

Washko, P. W., W. O. Hartzell, and M. Levine. (1989) Ascorbic acid analysis using high-performance liquid chromatography with coulometric electrochemical detection. Analyt. Biochem. 181: 276-282.

Washko, P. W., R. W. Welch, K. R. Dhariwal, Y. Wang, and M. Levine. (1992) Ascorbic acid and dehydroascorbic acid analyses in biological samples. Analyt. Biochem. 204: 1-14.

Fay, M. J., M. J. Bush, and A. J. Verlangieri. 1994. Effect of aldonic acids on the uptake of ascorbic acid by 3T3 mouse fibroblasts and human T lymphoma cells. General Pharmacology 25: 1465-1469.

Verlangieri, A. J., M. J. Fay, and A. W. Bannon. 1991. Comparison of the anti-scorbutic activity of L-ascorbic acid and Ester C in the non-ascorbate synthesizing Osteogenic Disorder Shionogi (ODS) rat. Life Sciences 48: 2275-2281.

Wright, J. V., and R. M. Suen. 1990. Comparative studies of "Ester C" versus L-ascorbic acid. International Clinical Nutrition Review : 267-271.

Bush, M. J., and A. J. Verlangieri. 1987. An acute study on the relative gastro-intestinal absorption of a novel form of calcium ascorbate. Research Communications in Chemical Pathology and Pharmacology 57: 137-140.

Bergsten, P., A. S. Moura, I. Atwater, and M. Levine. 1994. Ascorbic acid and insulin secretion in pancreatic islets. Journal of Biological Chemistry 269: 1041-1045.

TABLE 1

**Ascorbate and threonate content of products used in this study.** Ascorbate and threonate content is shown below as percentage by weight of each product.

| Product | Ascorbate Method | Ascorbate Assay (%) | Threonate Assay (%) |
|---|---|---|---|
| Ascorbic acid[a] | Iodometry | 99.6 | 0 |
| Ester-C calcium ascorbate[b] | Iodometry | 73.1 | 1.04 |
| Ester-C calcium ascorbate standardized with 3% threonate[c] | Iodometry | 71.0 | 3.0 |

TABLE 2A

**Group matching and vital statistics of subjects completing the study.** 54 candidate subjects were assigned randomly to one of 3 initial treatment groups (see text). Values for subjects completing the study are mean ± standard deviation. No significant differences were found between the 3 groups with respect to the 3 variables listed here.

| Group | N | Age (yr) | Weight (kg) | BMI* |
|---|---|---|---|---|
| Ascorbic acid | 18 | 31.8 ± 6.5 | 80.4 ± 11.2 | 25.3 ± 2.9 |
| Ester-C | 17 | 31.9 ± 6.3 | 82.7 ± 12.1 | 25.6 ± 2.5 |
| Ester-C plus threonate | 18 | 31.7 ± 7.2 | 80.8 ± 17.7 | 24.8 ± 4.7 |

* BMI = body mass index = weight divided by the square of height, given weight in kilograms and height in meters.

TABLE 2B

**Group matching and vital statistics of subjects completing the study (continued).** 54 candidate subjects were assigned randomly to one of 3 initial treatment groups (see text). Values for subjects completing the study are mean ± standard deviation. No significant differences were found between the 3 groups with respect to the 3 variables listed here.

| | | Fraction of Subjects | | |
|---|---|---|---|---|
| Group | N | Smokers | Supplement Users | Exercisers |
| Ascorbic acid | 18 | 0.12 ± 0.33 | 0.35 ± 0.49 | 0.88 ± 0.33 |
| Ester-C | 17 | 0.06 ± 0.24 | 0.22 ± 0.43 | 0.83 ± 0.38 |
| Ester-C plus threonate | 18 | 0.11 ± 0.32 | 0.39 ± 0.50 | 0.89 ± 0.32 |

TABLE 3

**Timing of Blood Collection.** Average time between ingestion of ascorbate supplement and collection of blood was computed for each of the 3 experimental groups at the target time points. Values are mean ± standard deviation for (N) subjects. No statistically significant differences were observed between corresponding time intervals.

| Group | Target Time (hr) | Elapsed Time (hrs) |
|---|---|---|
| Ascorbic Acid | 1 | 0.95 ± 0.08 (18) |
| | 2 | 1.96 ± 0.08 (18) |
| | 4 | 3.95 ± 0.08 (18) |
| | 24 | 23.97 ± 0.12 (18) |
| Ester-C | 1 | 0.96 ± 0.07 (17) |
| | 2 | 1.95 ± 0.10 (17) |
| | 4 | 3.95 ± 0.06 (17) |
| | 24 | 23.96 ± 0.10 (17) |
| Ester-C plus threonate | 1 | 0.96 ± 0.05 (18) |
| | 2 | 1.97 ± 0.06 (18) |
| | 4 | 3.93 ± 0.06 (18) |
| | 24 | 23.95 ± 0.06 (18) |

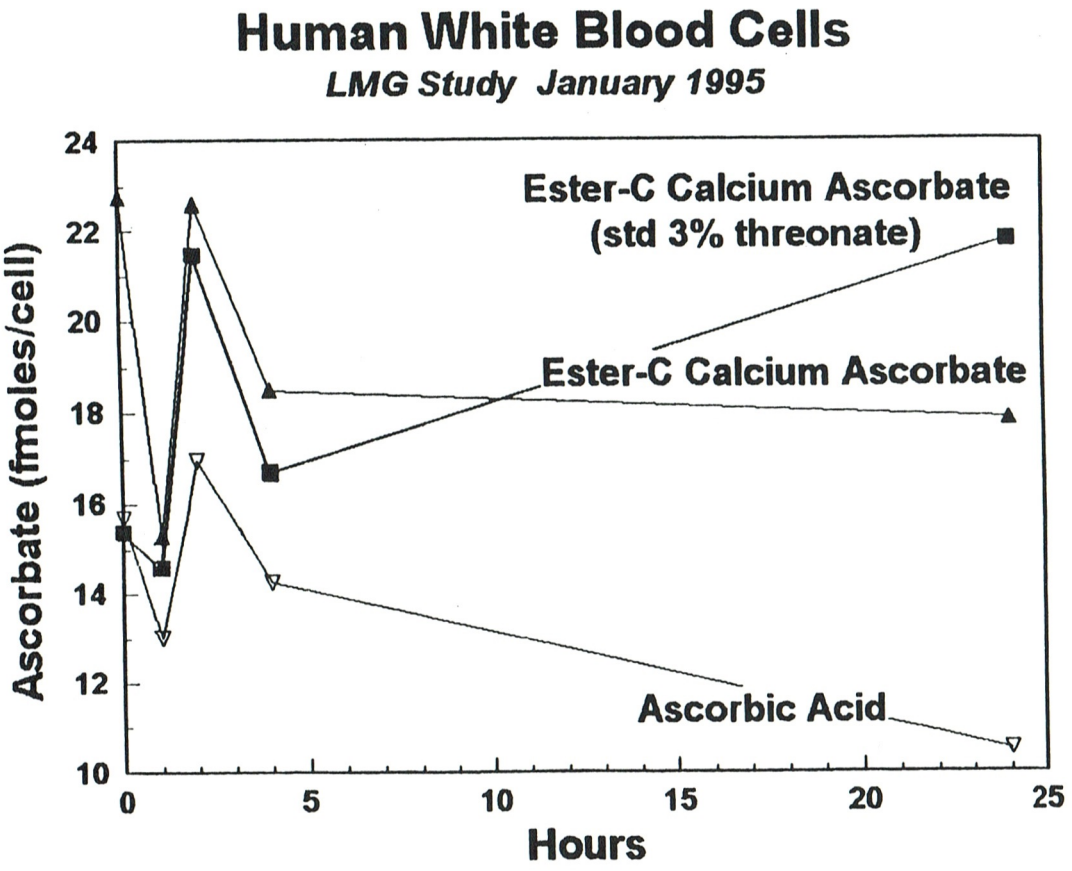



TABLE 4

**White blood cell ascorbate concentrations in test groups.** Target is the approximate elapsed time between ingestion of a single dose of ascorbate in product form (1000 mg) and blood collection. Values are mean ± standard deviation for (N) subjects for whom valid data were available. Values sharing a common lowercase letter in the rightmost column are significantly different at the 95% confidence level ($2P < 0.05$), according to a 2-tailed t-test. Only values at comparable timepoints were compared.

| Group | Target Time (hr) | WBC Ascorbate (fmoles/cell)* | | | Signif. Diff. |
|---|---|---|---|---|---|
| Ascorbic Acid | 0 | 15.69 ± | 9.46 | (16) | |
| | 1 | 13.02 ± | 4.57 | (17) | |
| | 2 | 16.96 ± | 12.22 | (18) | |
| | 4 | 14.25 ± | 9.73 | (18) | |
| | 24 | 10.52 ± | 5.57 | (17) | a |
| Ester-C | 0 | 22.76 ± | 17.94 | (16) | |
| | 1 | 15.30 ± | 7.75 | (17) | |
| | 2 | 22.60 ± | 20.73 | (14) | |
| | 4 | 18.50 ± | 35.18 | (16) | |
| | 24 | 17.85 ± | 7.79 | (14) | a |
| Ester-C plus 3% threonate | 0 | 15.37 ± | 10.88 | (15) | |
| | 1 | 14.58 ± | 9.36 | (18) | |
| | 2 | 21.45 ± | 20.89 | (18) | |
| | 4 | 16.68 ± | 31.23 | (18) | |
| | 24 | 21.77 ± | 31.77 | (16) | |

* White blood cell ascorbate calculated as femtomoles ($10^{-15}$ moles) per cell.

# Evaluation of the Tooth Erosive Characteristics of Ester-C-Zn

Grayson Marshall, DDS, MPH, PhD
Larry Watanabe
Dept. of Restorative Dentistry
Univ. of California, San Francisco, CA 94143-0758

## Introduction

A newly developed vitamin C preparation, Ester-C-Zn (EC-Zn), which the manufacturer indicates has a near neutral pH, is hypothesized to offer a number of advantages over ascorbic acid. One of the hypotheses is that EC-Zn induces less erosion of oral hard tissues than ascorbic acid. This hypothesis was tested as outlined below. For the initial evaluation we focused on the effect of EC-Zn on tooth enamel. It should be noted that effects on other oral hard tissues, cementum and dentin, might be greater than on enamel, but these tissues are often less exposed in normal human populations. Future studies might be expanded to include these other tissues, and the in vivo effects EC-Zn.

## Materials and Methods

An *in vitro* test of the erosive characteristics of two solutions, 2% EC-Zn (92mM ascorbic acid) and 2%; ascorbic acid (100 mM ascorbic acid), were carried out on blocks of human dental enamel. Six blocks of enamel were prepared as follows. Six mandibular incisors were used as they provided a relatively flat labial surface. The coronal portion of each tooth was sectioned at the cementum-enamel junction (CEJ) with a low speed water cooled diamond saw (Bueler Ltd.). The crown was then potted in cold curing resin (polymethyl methacrylate) with the labial surface exposed. The sample was then lightly ground to provide a metallograhically flat surface using 240 grit wet carborundum paper to provide a flattened area of approximately 5 mm diameter. A titanium alloy pin ('max 021', Whaledent Int.) was drilled and tapped in place in the center of the exposed enamel. The pin was cut flush with the surface,and the entire surface metallographically polished through a slurry 0.05 µm alumina. This provided a flat polished surface with a reference pin which served as a constant height reference throughout the measurements.

The specimens were randomly divided into two groups and periodic evaluations of the effect of exposure to either the 2% EC-Zn or 2% ascorbic acid solutions. During the exposure period the solution temperature was maintained near 37 ° C to simulate body temperature. Measurements were made after the following exposure times: 0 (baseline) 1, 2, 5, 10, 20, 50 minutes and 24 hours. At each time period, the samples were evaluated for changes in the enamel by various techniques: 1) direct measurement of depth differences between the titanium reference pin and the surrounding enamel; 2) pH and specific ion measurements of the solutions; and 3) SEM observation of enamel microstructure.

For the depth measurements, the titanium reference pin was taken as the center and zero point, and measurements relative to the pin height were made at 4 points located 1 mm radially from the pin and at 90 ° angular rotations from point to point. The device used to locate each measuring position was a precision X-Y micrometer and depth measurements were made with

a Tit Indicator micrometer accurate to 0.001 mm (Mitutoyo Mfg Co, Ltd.)

Solution characteristics of pH and specific ion (Ca) were made using an Accumet 1003 specific ion electrode (Fisher Scientific) with temperature compensation. This allowed for dissolved calcium and pH to be monitored periodically over the same time sequence as the other measurements. It should be noted that the Ca ion measurements are unreliable for the EC-Zn solutions, because Zn ions are a major interference for the measurement of Ca ions. Thus the ion concentration measured in the EC-Zn solutions are almost certainly Zn ions and not Ca ions.

Surface morphology was evaluated by scanning electron microscopy at each time period. For this portion of the study, we used a wet SEM (Modified SX-40A Wet SEM, Topcon Int.) This type of SEM permits observation of wet and/or uncoated specimens by providing a low vacuum (100 mTorr) which prevents charge build up. This allows repeated measurements of the same sample to be made at each step of the exposure process. At the conclusion of the experiment, the samples were cross sectioned through the pin and along one line of observation so that the profile of depth loss could be directly seen by SEM.

## Results and Discussion

Depth Changes

Table 1 below shows the depth changes relative to the titanium reference pin for each solution over the study period. Changes were too small for measurement at the 1 and 2 minute exposure periods and these values are indicated as not measurable (NM).

Table 1. Depth Changes (mm)

| Time (min) | n | Ascorbic Acid | n | EC-Zn | p-value |
|---|---|---|---|---|---|
| 1 | 12 | NM | 12 | NM | NS |
| 2 | 12 | NM | 12 | NM | NS |
| 5 | 12 | 0.002 (0.002) | 12 | 0.002 (0.005) | NS |
| 10 | 12 | 0.002 (0.003) | 12 | 0.003 (0.006) | NS |
| 20 | 12 | 0.006 (0.003) | 12 | 0.005 (0.006) | NS |
| 50 | 12 | 0.010 (0.002) | 12 | 0.002 (0.006) | .0001 |
| 1440 | 12 | 0.042 (0.015) | 12 | 0.004 (0.004) | <.00001 |

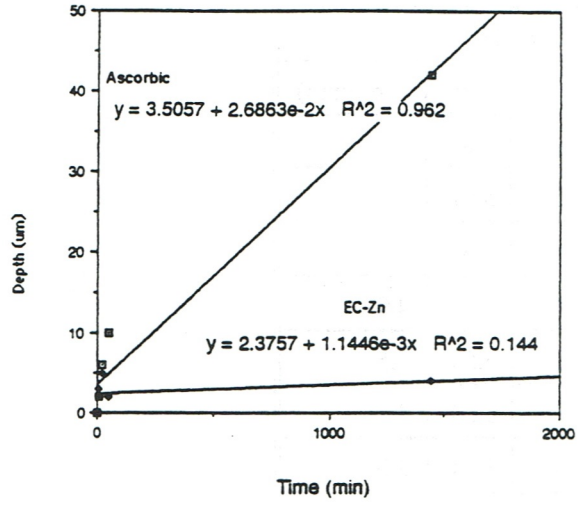
NM = not measurable; (SD) =standard deviation; NS= not significant

The depth changes at later times are significantly different, with the EC-Zn showing essentially no change, and the ascorbic acid causing increasing erosion.

The experiment also can be used to give an idea of the rate of the erosion from a regression of

depth vs time. Such a regression (Fig. 1) suggests that the ascorbic acid erodes the enamel surface at a rate of 0.03 μm/min ($r = .982$, $p < 0.001$), while the EC- Zn, produces a rate that is not significantly different than zero.

Fig. 1. Depth Change of Enamel vs Time for Ascorbic Acid and EC-Zn




The data at short exposure times were not accurate because the rate of erosion was below the detectibility limit for the technique. Should it be desirable to determine or compare early rates, atomic force microscopy would be an ideal and accurate method for these measurements since it is allows height measurements on the nanometer (nm) scale (Marshall et al, 1993).

pH and Specific Ion Concentration

The data are shown in Table 2 below.

Table 2. pH and Ion Concentration

| | Ascorbic Acid | | | Ester C-Zinc | | |
|---|---|---|---|---|---|---|
| | Sample | | | Sample | | |
| Time | | pH | Ion (ppm) | | pH | Ion (ppm) |
| 0 | 1 | 2.65 | | 4 | 5.61 | |
| | 2 | 2.64 | | 5 | 5.61 | |
| | 3 | 2.66 | | 6 | 5.61 | |
| 1 min | 1 | 2.66 | .016 | 4 | 5.55 | 4.9 |
| | 2 | 2.63 | .020 | 5 | 5.53 | 1.5 |
| | 3 | 2.65 | .011 | 6 | 5.60 | 4.7 |
| 2 min | 1 | 2.63 | .038 | 4 | 5.24 | 2.0 |
| | 2 | 2.63 | .024 | 5 | 5.25 | 2.2 |
| | 3 | 2.65 | .029 | 6 | 5.38 | 1.7 |
| 5 min | 1 | 2.62 | 1.1 | 4 | 4.91 | 9.2 |
| | 2 | 2.65 | .93 | 5 | 4.92 | 7.0 |
| | 3 | 2.64 | .32 | 6 | 4.92 | 7.2 |
| 10 min | 1 | 2.66 | .20 | 4 | 5.15 | 3.1 |
| | 2 | 2.65 | .12 | 5 | 5.12 | 4.6 |
| | 3 | 2.63 | .086 | 6 | 5.11 | 4.7 |
| 20 min | 1 | 2.66 | .61 | 4 | 5.01 | 2.9 |
| | 2 | 2.65 | .20 | 5 | 5.02 | 3.8 |
| | 3 | 2.65 | .18 | 6 | 5.05 | 3.1 |
| 50 min | 1 | 2.66 | .61 | 4 | 4.99 | 1.7 |
| | 2 | 2.67 | .74 | 5 | 4.92 | 3.5 |
| | 3 | 2.69 | .55 | 6 | 4.93 | 2.6 |
| 1440 min | 1 | 3.14 | 130 | 4 | 4.57 | 20.0 |
| | 2 | 3.08 | 110 | 5 | 4.59 | 15.0 |
| | 3 | 3.24 | 180 | 6 | 4.58 | 12.0 |

As noted previously Zn is an interfering ion for the detection of Ca by the specific ion probe,

and therefore the relatively high values for ion concentration in EC-Zn are likely a result of measurement of the Zn ion rather than Ca from tooth dissolution. For ascorbic acid the Ca ion concentration rose steadily as would be expected from the other work. The most meaningful difference between the solutions stems from the pH differences, which are about 2.6 for ascorbic acid and approximately 5 for EC-Zn. The ascorbic acid pH is sufficiently low to easily demineralize and erode the enamel surface (or other tooth surfaces), while EC-Zn has a pH sufficiently high that no significant dissolution took place.

SEM Morphology

The microscopic comparison shows the dramatic differences in the effects of ascorbic acid and EC-Zn on tooth enamel. In fact clear differences are easily observed with the unaided eye. The baseline polished surfaces had a high polish. This polished appearance remained throughout testing with EC-Zn, but was rapidly altered by the ascorbic acid and took on a frosty white appearance which is typical of enamel which has been acid etched (Marshall et al, 1975; 1988). Such an appearance is used in clinical situations to indicate a sufficiently good etch has been achieved for bonding pit and fissure sealants and dental composites in restorative dental procedures.

The figures below show the morphology of the samples at various time periods. Clearly, the ascorbic acid samples took on the typical etched appearance at the earliest time periods, while the EC-Zn treatment had no noticeable effect on the enamel. Our conclusion from the morphological study is that ascorbic acid immediately and continuously eroded the enamel, while the EC-Zn solution had no effect.

The figures show the change in morphology which occurred at each exposure period. Figure 2 shows the polished baseline surface for samples used for the EC-Zn and ascorbic acid exposures, respectively. A few polishing scratches are apparent.

Fig. 2a. Baseline polished surface

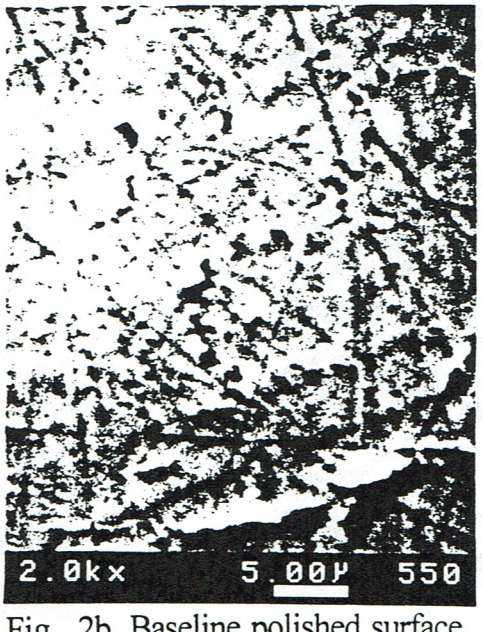



Fig. 2b. Baseline polished surface.

Fig. 3 shows exposure periods at 1 minute for EC-Zn in a) and ascorbic acid in b). Enamel etching patterns are present for ascorbic acid and some remnants of polishing scratches are seen, but essentially no change occurred for EC-Zn exposure. At 2 minutes, a fully developed etching patterns were present for ascorbic acid (Fig. 4a) while no changes could be seen for EC-Zn exposure (Fig. 4b). This pattern continued with little difference though 50 minute exposure (see Appendix). At 24 hours, the structure was deeply etched and erroded for ascorbic acid (Fig. 5a), while no change was apparent in the surface after EC-Zn exposure (Fig. 5b).

Fig. 3a. 1 min. ascorbic acid.

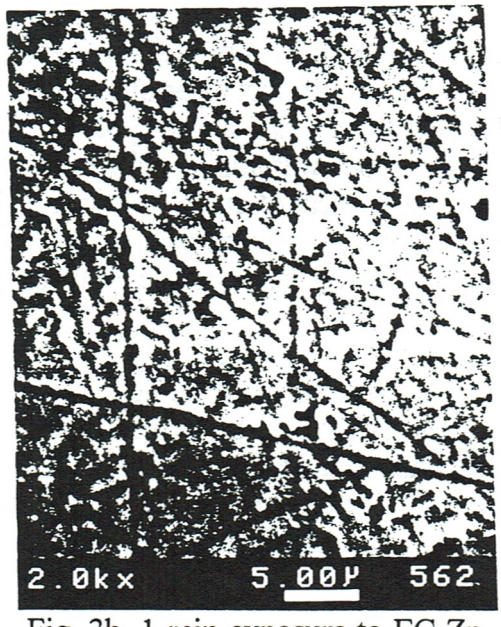



Fig. 3b. 1 min exposure to EC-Zn.

Fig.4a. 2 min. ascorbic acid.

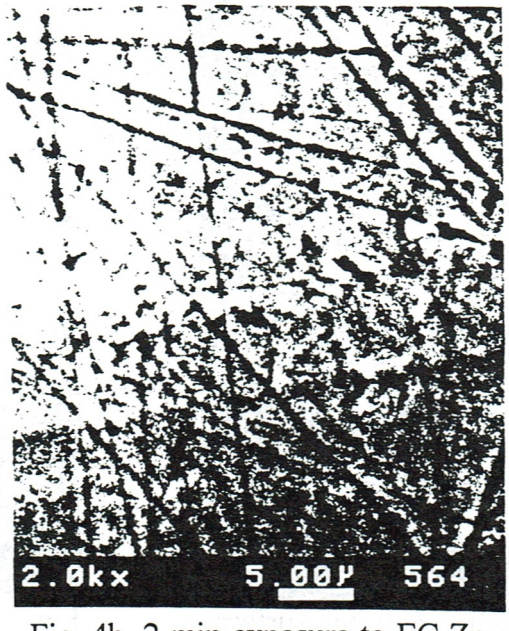



Fig. 4b. 2 min exposure to EC-Zn.

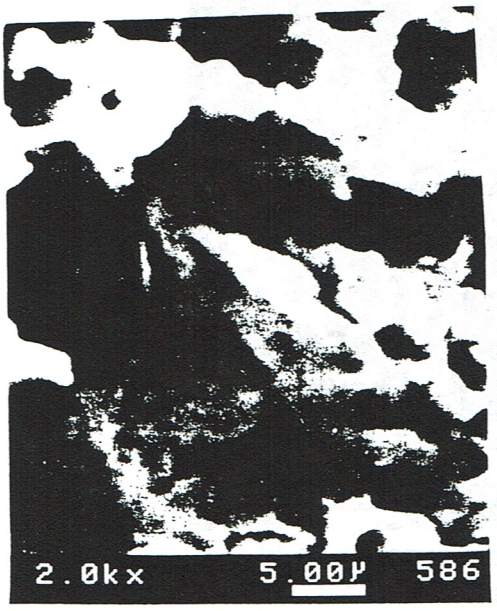



Fig.5a. 24 hrs. ascorbic acid.

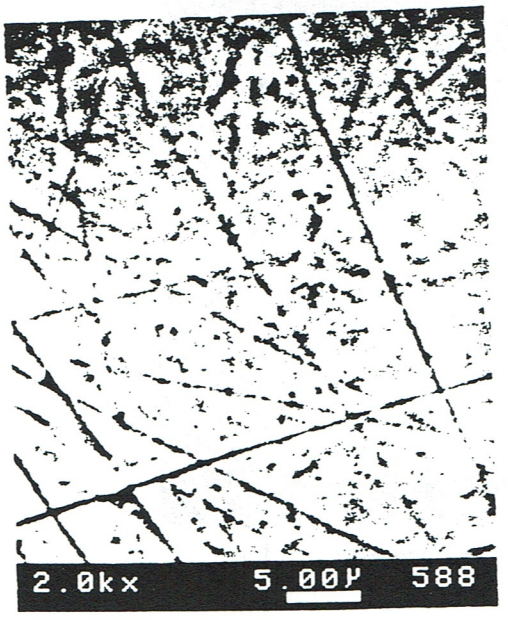



Fig. 5b. 24 hrs. exposure to EC-Zn.

At the completion of the erosion experiments, the samples were cross sectioned through the reference pin. The cross sections are shown in Fig.6-7.

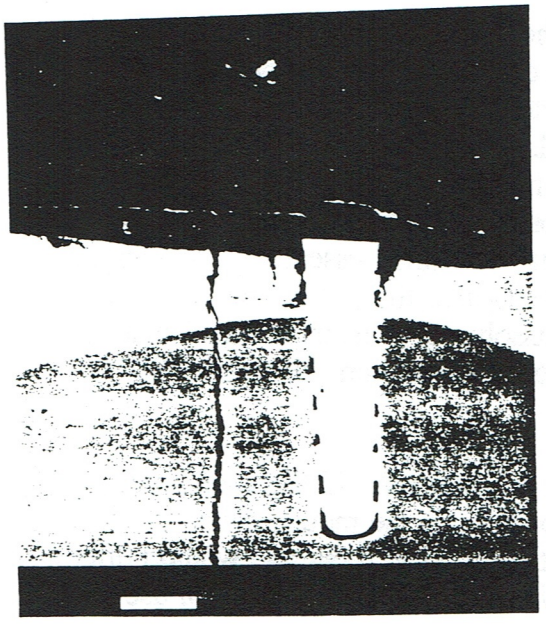


Fig.6a. 24 hrs. ascorbic acid.
20 X of cross section through reference pin.

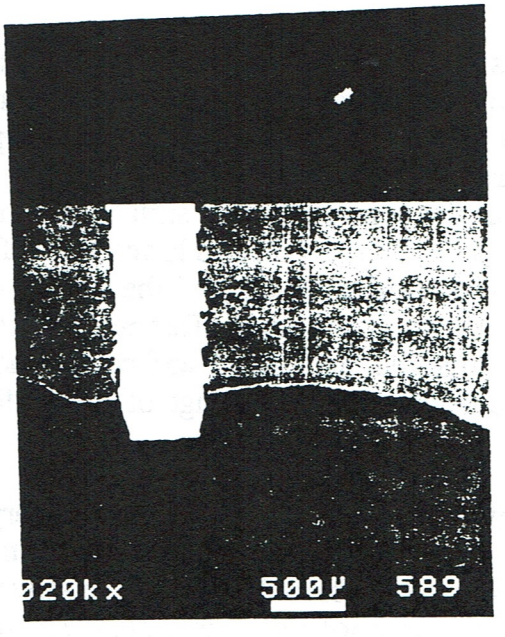



Fig. 6 b. 24 hrs. exposure to EC-Zn
20 X of cross section through reference pin.

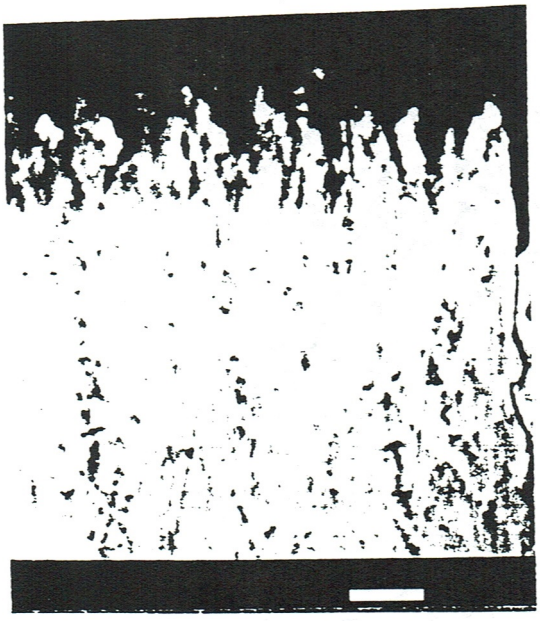


Fig.7a. 24 hrs. ascorbic acid. Higher magnification with deep etching of enamel.

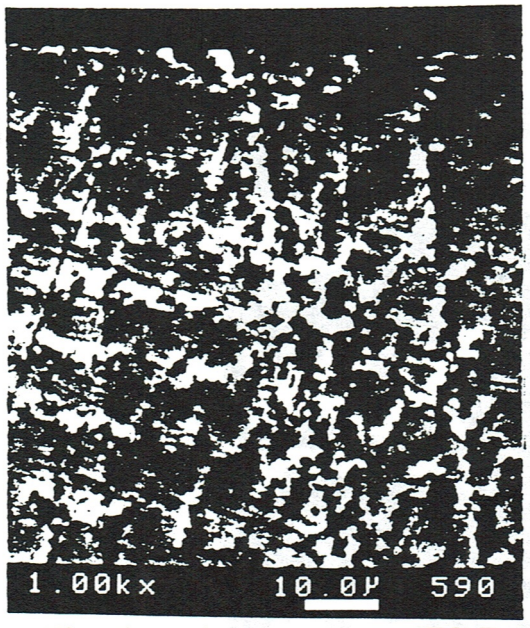



Fig. 7b. 24 hrs. exposure to EC-Zn Higher magnification with no no etching of enamel.

The severe erosion of enamel surface relative to the pin is clearly shown for ascorbic acid. In contrast with this result, the pin surface and enamel surface are at the same level for the EC-Zn exposure. This confirms the other observations of significant erosion with ascorbic acid and the lack of erosion with EC-Zn.

**Summary**

The various results obtained in the study were consistent. The pH of ascorbic acid is sufficiently low to dissolve the enamel surface which results in continued erosion throughout the experiments. This dissolution was immediately apparent as typical acid etching patterns showing the enamel rod structure developed in the ascorbic acid as seen by SEM. This effect is also apparent to the naked eye as the polished enamel surface becomes frosty white and dull after the ascorbic acid exposure, but retained its polished luster for the EC-Zn exposure. For the EC-Zn solution, there was no significant dissolution, and the surface of the enamel remained essentially unchanged during the course of the study. This result is to be expected since the pH of the solution is sufficiently high that the enamel surface does not readily dissolve. It is generally held that the pH must be below the critical range of 4.5-5.5 for such enamel dissolution (Menaker, 1980).

A series of human studies would be necessary in order to determine if the magnitude of differences seen in these *in vitro* experiments, might also be seen in the mouth. Since the oral cavity is continuously bathed in saliva, the saliva acts as both a diluting agent and would buffer any vitamin C prepartion introduced into the mouth. Variables such as dosage, length of use and methods of use should also be considered.

In conclusion acorbic acid dissolved enamel "*in vitro*", whereas EC-Zn did not.

## References

Marshall GW, Olson LM, Lee CV. SEM Investigation of the Variability of Enamel Surfaces after Simulated Clinical Acid Etching for Pit and Fissure Sealants. J. Dent. Res. 54:1222-1231, 1975.

Marshall GW, Marshall SJ, Bayne SC. Restorative Dental Materials: Scanning Electron Microscopy and X-ray Microanalysis. Scanning Microsc. 2:2007-2028, 1988.

Marshall GW, Balooch M, Tench R, Kinney JH, Marshall SJ. Atomic Force Microscopy of Acid Effects on Dentin. Dent Mater 9:265-268, 1993.

Menaker L, Editor The Biologic Basis of Dental Caries, Harper and Row Publisher, Hagerstown MD, 1980.

## Appendix. Additonal SEM Comparison of Errosion Patterns

Fig.8a. 5 min. ascorbic acid.

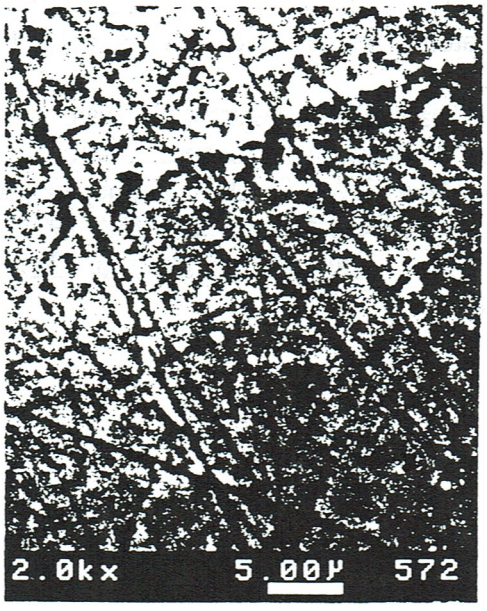



Fig. 8b. 5 min exposure to EC-Zn.

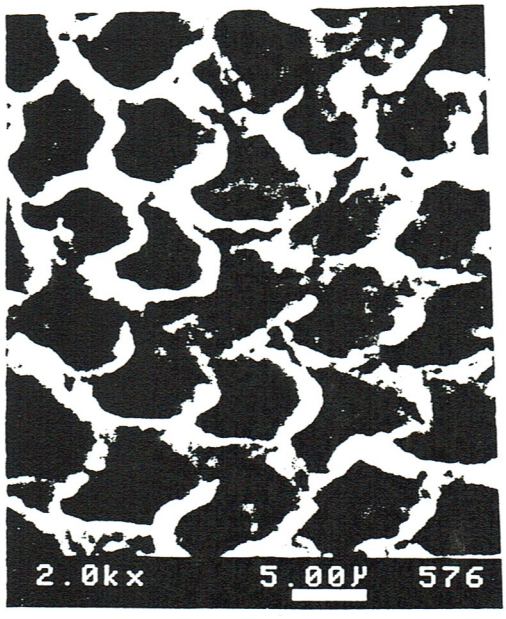



Fig. 9a. 10 min. ascorbic acid.

Fig. 9b. 10 min exposure to EC-Zn.

Fig. 10a. 20 min. ascorbic acid.

Fig. 10b. 20 min exposure to EC-Zn.

Fig. 11a. 50 min. ascorbic acid.

Fig. 11b. 50min exposure to EC-Zn.

PRE-PUBLICATION MANUSCRIPT
FOR INFORMATION ONLY
DO NOT REFERENCE

DRAFT

January 3, 1995

# The Antimicrobial Capacity of Zinc Ascorbate

Steven J. Silverstein, DMD, MPH
Professor
Department of Dental Public Health & Hygiene
University of California
School of Dentistry

Charles I. Hoover, Ph.D.
Assistant Adj. Professor
Department of Stomatology
University of California
School of Dentistry

There is now an extensive literature which demonstrates that the daily use of toothpastes and mouthrinses that incorporate antiplaque agents improves oral health.(1,2) Zinc is one of these antimicrobial which has clinical antiplaque activity.(3,4) Zinc is also highly retained in the oral cavity.(5,6) There are two zinc compounds that have antiplaque activity and are currently used in dental products. These zinc compounds are formulated with other active antiplaque ingredients. The first is zinc chloride which is found in both an antiplaque toothpaste and mouthrinse.(7) The second is zinc citrate trihydrate which is present in a dentifrice.(8)

Ascorbic acid is the one of the most potent antioxidants. However, the therapeutic use of ascorbic acid in dentistry has been limited. One study showed that gingival bleeding increases after a period of ascorbic acid depletion and returns to normal after ascorbic acid repletion.(9) Another study showed that smokers and non-smokers who took a twice a day vitamin C supplement of 500 mg for one month had a decrease in crevicular fluid flow compared to a control group.(10) In another approach researchers combined ascorbic acid with chitosan to form a chitosan ascorbate gel which was placed on periodontal surgery sites. Biopsies taken on 10 patients showed proliferation and organization of cells and subsequent

enhanced capability of reconstruction of the histoarchitectural tissue.(11) In a study reported in General Dentistry (Nov/Dec. 1993), patients receiving 1500 mg of vitamin C had a higher percentage of faster healing than placebo.

Another limiting factor of the use of ascorbic acid in the oral cavity is the drop in salivary pH below 5.1 while dissolving vitamin C containing tablets. The repeated use of chewable megadose tablets as a mint can damage teeth by dissolving the enamel.(12)

This study was undertaken to investigate the "in vitro" antiplaque activity of zinc ascorbate (Ester C {R}). Ester C (U.S. patent no. 4822816, Inter-Cal Corporation, Prescott, AZ) is a complex mixture containing zinc ascorbate, dehydroascorbate, zinc threonate, and zinc oxide. A 1% solution of Ester C has a pH of 7.1 and has been shown to be more rapidly absorbed and excreted less rapidly in rats than ascorbic acid.(13) Another study by Fay and Verlangieri have shown that calcium threonate facilitates the uptake of C(14)-ascorbic acid into T-cell lymphoma cells in culture.(14)

## Materials and Methods

(1) Calculation of $Zn^{++}$ as a percentage of Ester-C Zinc

| | |
|---|---|
| Zinc from ascorbate | 12.4% |
| Zinc form threonate | 1.3% |
| Zinc from oxide | 2.1% |
| **Ester-C Zinc** | **15.8% $Zn^{++}$** |

(2) Calculation of amount of Ester-C Zinc that contains 50 mM $Zn^{++}$

1M $Zn^{++}$ = 65.4 g/L; 1mM = 0.0654 g/L;
50mM = 3.27 g/L; 50mM = 0.327 g/100 ml;
**50mM = 0.1624 g/50ml**

- Amount of Ester-C Zinc that contains 0.1635 g $Zn^{++}$

0.1635/0.158 = 1.03 g;
**1.03 g Ester-C Zinc dissolved in 50 ml water = 50 mM $Zn^{++}$**

(3) 2X Stock Ester-C Zinc (2% Ester-c Zinc = 50 mM $Zn^{++}$)

Dissolve 1.03 g Ester-C Zinc in 50 ml distilled water
Filter-sterilize with 0.2 um membrane filter

Ester-C Zinc was not completely soluble, insoluble material was trapped on the membrane filter, pH of 2% Ester-C Zinc was 6.2

(4) 2X Stock $ZnCl_2$ (0.66% $ZnCl_2$ = 50 mM $Zn^{++}$)
Dissolve 0.33 g $ZnCl_2$ in 50 ml distilled water
Filter-sterilize with 0.2 um membrane filter
pH was 6.4 (5) 2X Stock L-ascorbic acid (100 mM L-ascorbic acid)*
Dissolve 0.88 g L-ascorbic acid in 50 ml distilled water
Initial pH was 2.6, adjusted to pH 6.6 with NaOH
Filter-sterilize with 0.2 um membrane filter
* 2X stock solution of Ester-C Zinc = 92 mM L-ascorbic acid

(6) Preparation of culture media containing various concentrations of the test compounds
For determining MIC values, two-fold serial dilutions of each of the test compounds (Ester-C Zinc, $ZnCl_2$, & L-ascorbic acid) were prepared in sterile trypticase soy broth supplemented with 0.25% yeast extract (sTSB). For each test compound the first two-fold serial dilution was made using 2X sTSB, subsequent two-fold serial dilutions were made with 1X sTSB. For determining PfIC values dilutions were made as described above with 2X sTSB containing 10% sucrose and 1X sTSB containing 5% sucrose. The concentrations of the test compounds used in the various assays are shown in the Results section.

Note: All stock solutions were prepared fresh immediately before each assay. Culture media containing Ester-C Zinc (1% & 0.5%) and $ZnCl_2$ (25mM & 12.5mM) exhibited some initial turbidity, presumably due to partial coagulation of protein components of the media.

(7) Growth of bacterial inoculum
*S. mutans* and *S. sobrinus* were cultured overnight in 5 ml trypticase soy broth (Difco Laboratories) containing 0.25% yeast extract. Two hundred ul of overnight culture was used to inoculate 5 ml of culture media containing the two-fold serial dilutions of each test compound.

(8) Scoring the results
The effect of the various concentrations of the test compounds on growth and plaque formation on glass *in vitro* were qualitatively determined after 24-48 h incubation at 37 C by comparison to grow or plaque formation obtained in sTSB or sTSB containing 55 sucrose alone (positive control). The MIC was defined as the lowest concentration of test compound that inhibited bacterial growth. The PfIC was defined as the lowest concentration of test compound that inhibited the formation of bacterial plaque on glass.

PRE-PUBLICATION MANUSCRIPT
FOR INFORMATION ONLY
DO NOT REFERENCE

## RESULTS

- = clear media/no growth: bacteria inhibited by test compound
+ = turbid media: bacteria not inhibited by test compound

*Streptococcus mutans* 25175

(A) Minimal Inhibitory Concentrations (MIC) scored as turbidity vs. control in sTSB

| Ester-C Zinc conc. | 1% | 0.5% | 0.25% | 0.125% | control |
|---|---|---|---|---|---|
| 12/08/93 | - | - | +++ | +++ | +++ |
| 12/16/93 | - | - | +++ | +++ | +++ |
| 01/07/94 | - | - | +++ | +++ | +++ |

| $ZnCl_2$ conc. | 25mM | 12.5mM | 6.25mM | 3.125mM | control |
|---|---|---|---|---|---|
| 12/08/93 | - | - | +++ | +++ | +++ |
| 12/16/93 | - | - | +++ | +++ | +++ |
| 01/07/94 | - | - | +++ | +++ | +++ |

| L-ascorbic acid conc. | 50mM | 25mM | 12.5mM | control |
|---|---|---|---|---|
| 12/08/93 | +++ | +++ | +++ | +++ |
| 12/16/93 | +++ | +++ | +++ | +++ |
| 01/07/94 | +++ | +++ | +++ | +++ |

(B) Plaque-forming Inhibitory Concentrations (PfIC) scored as adherence to glass as compared to control in sTSB containing 5% sucrose

| Ester-C Zinc conc. | 1% | 0.5% | 0.25% | 0.125% | control |
|---|---|---|---|---|---|
| 01/13/94 | - | - | +++ | +++ | +++ |
| 01/19/94 | - | - | +++ | +++ | +++ |

| $ZnCl_2$ conc. | 25mM | 12.5mM | 6.25mM | 3.125mM | control |
|---|---|---|---|---|---|
| 01/13/94 | - | - | +++ | +++ | +++ |
| 01/19/94 | - | - | +++ | +++ | +++ |

| L-ascorbic acid conc. | 50mM | 25mM | 12.5mM | control |
|---|---|---|---|---|
| 01/13/94 | +++ | +++ | +++ | +++ |
| 01/19/94 | +++ | +++ | +++ | +++ |

PRE-PUBLICATION MANUSCRIPT
FOR INFORMATION ONLY
DO NOT REFERENCE

*Streptococcus sobrinus* 6715

(A) Minimal Inhibitory Concentrations (MIC) scored as turbidity vs. control in sTSB

| **Ester-C Zinc conc.** | **0.125%** | **0.06125%** | **0.03%** | **0.015%** | **control** |
|---|---|---|---|---|---|
| 01/13/94 | - | - | +++ | +++ | +++ |
| 01/19/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |

| **$ZnCl_2$ conc.** | **3.125mM** | **1.625mM** | **0.8mM** | **0.4mM** | **control** |
|---|---|---|---|---|---|
| 01/13/94 | - | - | +++ | +++ | +++ |
| 01/19/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |

| **L-ascorbic acid conc.** | **50mM** | **25mM** | **12.5mM** | **control** |
|---|---|---|---|---|
| 01/13/94 | +++ | +++ | +++ | +++ |
| 01/19/94 | +++ | +++ | +++ | +++ |
| 01/20/94 | +++ | +++ | +++ | +++ |

(B) Plaque-forming Inhibitory Concentrations (PfIC) scored as adherence to glass as compared to control in sTSB containing 5% sucrose

| **Ester-C Zinc conc.** | **0.125%** | **0.06125%** | **0.03%** | **0.015%** | **control** |
|---|---|---|---|---|---|
| 01/19/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |

| **$ZnCl_2$ conc.** | **3.125mM** | **1.625mM** | **0.8mM** | **0.4mM** | **control** |
|---|---|---|---|---|---|
| 01/19/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |
| 01/20/94 | - | - | +++ | +++ | +++ |

| **L-ascorbic acid conc.** | **50mM** | **25mM** | **12.5mM** | **control** |
|---|---|---|---|---|
| 01/19/94 | +++ | +++ | +++ | +++ |
| 01/20/94 | +++ | +++ | +++ | +++ |
| 01/20/94 | +++ | +++ | +++ | +++ |


## SUMMARY

Minimal inhibitory concentrations (MIC) and plaque-formation inhibitory concentrations (PfIC) for Ester-C Zinc and $ZnCl_2$ were determined using *S. mutans* 25175 and *S. Sobrinus* 6715. The MIC determined for *S. sobrinus* 6715 was 0.5% Ester-C Zinc and 12.5 mM

$ZnCl_2$, therefore *S. sobrinus* 6715 appears to be approximately eight times more sensitive to these compounds than *S. mutans* 25175. With both strains inhibition of plaque formation (PfIC) occurred at the same concentration that inhibited growth ( the MIC).

L-ascorbic acid at concentrations equivalent to those in the Ester-C Zinc solutions tested had no antimicrobial effect. Since the MIC values obtained with Ester-C Zinc parallel those obtained with equivalent concentrations of $ZnCl_2$, the antimicrobial effect of Ester-C Zinc is most likely due to its zinc content.

Further studies could determine if Ester-C Zinc has bactericidal activity in addition to the inhibitory (bacteriostatic) effect demonstrated by these studies. If bactericidal activity is detected it would be of interest to determine the exposure time required for Ester-C Zinc to exert bacterial killing. Determining the sensitivity of addition strains of *S. mutans* and *S. sobrinus* to Ester-C Zinc might also be useful to determine which of the two strains tested is more typical of oral streptococcal strains associated with dental caries.

PRE-PUBLICATION MANUSCRIPT
FOR INFORMATION ONLY
DO NOT REFERENCE

## REFERENCES


1. Svatun B, Saxton C A, Huntington E et al. The effects of three silica dentifrices containing Triclosan on the supra-gingival plaque and calculus formation and on gingivitis. Int Dent J 1993 43:441-452.

2. Van der Ouderaa F G. Human clinical studies of antiplaque and antigingivitis agents dosed from dentifrice. In: Clinical and Biological Aspects of Dentifrices. G Embery and G Rolla (eds.) pp. 181-204. Oxford University Press, 1992.

3. Hancke T M. Studies of the local factors on dental caries. 1. Destruction of plaque and retardation of bacterial growth in the oral cavity. J Amer Dent Assoc 1940 27:1379-1393.

4. Watson G K, Cummins D. Importance of speciation of zinc to its antimicrobial activity. (Abstract). J Dent Res 1988 67:2314.

5. Harrap G J, Best J S, Saxton C A. Human oral retention of zinc from mouthwashes containing zinc salts and its relevance to dental plaque control. Archs Oral Biol 1984 29:87-91.

6. Saxton C A, Cummins D. The effect of zinc citrate dentifrices on the development of gingivitis (Abstract) J Dent. Res 1989 68 (Special Issue): 971.

7. Eisenberg A D, Young D A. Interactions of sanguinarine and zinc on oral streptococci and actinomyces species. Caries Res 1991 25(3): 185-90.

8. Svatun B, Saxton C A, Roola G. Six month study of the effect of a dentifrice containing zinc citrate and Triclosan on plaque, gingival health, and calculus. Scand J Dent Res 1990 98:301-304.

9. Leggott P J, Robertson P B, Jaco J J et al. Effects of Ascorbic Acid depletion and supplementation on periodontal health and subgingival microflora in humans. J Dent Res 1991 70 (12): 1531-1536.

10. Holmes L G. Effects of smoking and/or vitamin C on crevicular fluid in clinically health gingiva. Quintessence Inter. 1990 21(3):191-95.

11. Muzzarelli R, Biagini G, Pugnaloni A et al. Reconstruction of periodontal tissue with chitosan. Biomaterials 1989 10(9):598-603).

PRE-PUBLICATION MANUSCRIPT
FOR INFORMATION ONLY
DO NOT REFERENCE

12. Hayes G L, Bullock Q, Lazzari et al. Salivary pH while dissolving vitamin C-containing tablets. Amer J. Dent. 1992 5(5):269-71.
13. Fay and Verlangieri
14. Fay and Verlangieri

# PROCEEDINGS©

# 1994 ANNUAL CONFERENCE
# ORLANDO, FLORIDA

# VITAMIN C (ASCORBIC ACID) - NEW FORMS AND NEW USES IN DOGS

L. Phillips Brown, D.V.M.

**Summary**

The effects of different forms of vitamin C on various locomotor dysfunctions of dogs were investigated. Over one hundred case studies at an animal sanctuary were evaluated using varying strengths and combinations of mineral ascorbates, vitamin C (ascorbic acid) and microcrystalline cellulose during a six month period. The results indicate that a patented vitamin C ascorbate/vitamin C metabolite complex administered orally may have application for the reduction of discomfort associated with chronic inflammatory disorders of the musculoskeletal system of dogs.

**Preliminary Discussion**

Vitamin C (ascorbic acid) is a vital nutrient required for many fundamental biosynthetic pathways. Ascorbic acid neutralizes free radicals (unpaired electrons that breakdown cell structures and promote aging and disease) and rejuvenates oxidized enzymes (Bielski et al. , 1975; Bodannes and Chan, 1979; Retsky et al. , 1993). Ascorbic acid may play a role in prevention and/or treatment of heart disease (Simon, 1992; Kozlenko, 1994), stimulation of the immune system (Prinz et al. , 1977; Anderson, 1981; Goldschmidt, 1991), reduction of cancer mortality or incidence (Block, 1991) and inhibition of viral activity (Harakeh et al. , 1990). Studies in dogs (Belfield, 1969; Berge, 1990; Docktor, 1983) and horses (Newman, 1994) suggest that vitamin C may aid the healing of joint disorders and in the regeneration of connective tissue. Vitamin C may also help counteract the effects of heavy exercise in working dogs (Kronfeld, 1988). Millions of people supplement their diets daily with vitamin C because humans cannot produce endogenous ascorbic acid, and the amounts derived from diets may not provide optimum levels. Dogs and cats, unlike primates, guinea pigs and certain other species, do have the necessary enzymes to convert glucose to ascorbic acid and should not require or benefit from vitamin C supplementation. Nevertheless, veterinary clinicians and researchers are beginning to question the ability of dogs and cats to produce sufficient quantities of endogenous ascorbic acid to counter the effects of aging, stress, breed predilections to inherited dysfunctions, environmental irritants and pollutants and poor quality or high fat pet foods.

Dogs are subject to numerous joint and spinal disorders, including hip dysplasia, osteoarthritis, non-specific arthritis, osteochrondritis, spondylitis and spondylosis. The severity of radiographic signs and the intensity of discomfort are directly related to the degree of articular cartilage or discoid deterioration. Treatment often consists of rest, surgery and/or steroids, nonspecific anti-inflammatory drugs (NSAIDS), aspirin, penicillamine or methotrexate. Many therapeutic compounds produce only short-term benefits and may actually accelerate the progression of joint destruction.

**Chondroregenerative Properties of Ascorbic Acid**

Ascorbic acid is important in maintaining the physical integrity of collagen. Cartilage consists of collagen, proteoglycans and water. Collagen is a tough, stringy protein that physically connects tissues and provides the tensile strength of cartilage. Proteoglycans trap and hold water, allowing for compression resistance. In normal cartilage, the anabolic and catabolic processes of chrondrocyte cells are in equilibrium. In degenerative or inflammatory conditions, the breakdown processes are excessive. Ascorbic acid (vitamin C) may serve:

1. As a reducing agent that promotes enzymatic hydroxylation of collagen and as a carrier of activated sulfates needed for the synthesis of glycosaminoglycans, one of the building blocks of proteoglycans. Vitamin C may retard the catabolic process by inhibiting arylsulfatase B, which desulfates glycosaminoglycans.
2. As an immunoresponsive agent against infectious organisms by enhancing phagocytic activity and modulating production of lymphocytes and mobilizing them to the site of infection. Vitamin C stimulates the synthesis of complement, production of interferon and regulation of prostaglandin synthesis.
3. As an antioxidant to prevent damaging effects of superoxides, peroxides, hydroxyl radicals and singlet oxygen molecules by neutralizing toxic oxygen generated by phagocytic activity.
4. As a regenerator of vitamin E radicals, thus allowing vitamin E to continue its role as a potent antioxidant.

**Materials and methods**

The study was conducted at Best Friends Animal Sanctuary in Kanab, Utah. Dogs were selected on the basis of a history of chronic lameness, decreased mobility and discomfort as reported by the sanctuary staff handlers. Study dogs were chosen from over nine hundred animals housed at the sanctuary. All study dogs were given an initial physical examination by veterinarians and examined daily by handlers and monthly by the sanctuary staff veterinarian. Each handler was assigned the care of specific dogs and administered a compound labeled only by code (see table 1) twice daily. Observations on the extent of change were recorded in a weekly log.

| Compound code | Composition |
|---|---|
| AIOI | 2000 mg vitamin C from Ester-C calcium ascorbate/t |
| A102 | 850 mg vitamin C from Ester-C calcium ascorbate/t |
| A103 | 2000 mg vitamin C from Ester-C Ca/Mg/K/Zn ascorbic It |
| A104 | 2000 mg vitamin C from ascorbic acid/t |
| A105 | Mcrocrystalline cellulose |

Table 1

The study was conducted in three phases. During Phase 1, each dog was given 1/4 teaspoon per 25 lb. (12 kg) body weight twice daily orally of one of the compounds listed in Table I for four weeks, followed by three weeks of no ministrations. During the second phase, each dog was given a compound different from the one given in phase I at the same dosage rate for the next four weeks, followed by another three weeks of no ministrations. During phase 111, some dogs were given the same compound received during phase 1, while others were given compounds they had not previously received (Table 2).

| Group | Phase I | Phase II | Phase III |
|---|---|---|---|
| I | A1O1 | A105 | A1O1 |
| II | A102 | A104 | A1O1 |
| III | A103 | A1O1 | A104 |
| IV | A104 | A102 | A1O1 |
| v | A105 | A1O1 | A105 |

Table 2

Response in all studies was measured by changes of comfort and activity level and graded by veterinarians, handlers and owners according to Table 3.

| Grade | Response |
|---|---|
| 0 | No Change |
| 1 | Slight/Mild Improvement |
| 2 | Moderate/Good Improvement |
| 3 | Very Good Mobility |

Table 3

**Ester-C ascorbate**

According to the manufacturer, Inter-Cal Corporation, of Prescott, Arizona, Ester-C is a patented calcium ascorbate supplement that contains calcium ascorbate, calcium, naturally occurring dehydroascorbate and the vitamin C metabolite, threonate. Threonate permits ascorbate to be more rapidly absorbed from the gastrointestinal tract, cross cell membranes more efficiently, reach higher cellular levels and be excreted more slowly than ordinary vitamin C (Fay, et al, 1991; Veriangieri, 1991). Ester-C ascorbate is pH neutral and believed to be water and partially fat soluble.

**Results**

Study results appear in tables 4A, 4B and 4C.

**Study Summary**

78% of the dogs receiving Ester-C calcium ascorbate (compound AIOI) at a dosage of 45 mg/kg body weight twice daily showed improved mobility within 4-5 days. The average extent of improvement (see note 1) was 1. 52 with a significant number of dogs rated "very good". Approximately 60% of these "improved" dogs showed renewed discomfort upon discontinuance of Ester-C calcium ascorbate and renewed mobility when it was administered during a different phase. No side negative effects were noted. Many handlers reported increased "playfulness" and "energy" of the dogs while on Ester-C ascorbate.

52% of the dogs showed improvement within 7 days of receiving Ester-C calcium ascorbate (compound A102) at a dosage of 22 mg/kg body weight twice daily. The average extent of improvement was 0. 45 with most dogs showing mild to moderate mobility improvements No side effects were noted. The level of response to compound Al 02 was not as pronounced as that seen with Ester-C calcium ascorbate at 45 mg/kg BID dose.

62% of the dogs receiving Ester-C multi-n-fineral ascorbate (compound A103) at a dosage of 45 mg/kg body weight twice daily showed locomotor improvement within 5-8 days. The average extent of improvement was 0. 87 with mild to good improvement. Approximately 40% of the "For further information, contact:improved" dogs experienced moderate discomfort upon discontinuance of the compound.

44% of dogs receiving ordinary vitamin C (ascorbic acid) at a dosage of 45 mg/kg body weight twice daily showed some improvment in locomotor ability. The average extent of improvement on compound A104 was 0. 67. Two dogs experienced transitory gastrointestinal disturbances.

4% of the dogs responded to microcrystalline cellulose (compound A105). The average extent of improvement was 0. 04. Any improvements on this compound could result from increased attention or observational subjectivity.

## Conclusion

The consistency and degree of response demonstrates that Ester-C calcium or mineral ascorbates provide symptomatic relief to dogs suffering from selected chronic joint and musculoskeletal disorders. The findings that the condition of many of the "improved" dogs deteriorated rapidly upon discontinuance of Ester-C ascorbate further verifies its usefulness as primary or adjunctive therapy. These studies reinforce the earlier work of Docktor and Berge, which earlier demonstrated some positive effects of supplemental Ester-C calcium ascorbate and its metabolites.

The observation that the response of many dogs to Ester-C at a dose of 45 mg/kg B. W. BID was better than the response to the same compound at 22 mg/kg indicates a dose response relationship exists. Although there was some individual improved function with ordinary vitamin C, percentages of case studies showing mobility improvements(table 4B was significantly lower than with Ester-C ascorbate.

The study findings suggest that, although dogs can manufacture endogenous ascorbic acid, the amount produced in some dogs may not be sufficient to prevent or counteract stresses associated with aging, injury or joint malpositioning. Low or declining ascorbic acid availability within chrondrocytes may compromise collagen homeostasis, resulting in loss of tissue elasticity and/or compression resistance. Clinical signs of stiffness, discomfort and/or decreased mobility may be further exaggerated by age, exercise, inflammation or improper nutrition. A form of vitamin C that would promote higher levels of intracelluar ascorbic acid without negative side effects. would be a useful alternative to steroids and aspirin.

From the reslults of this study, there is significant evidence to recommend oral Ester-C ascorbate in the treatment of non specific musculoskeletal disorders of dogs.

note 1: The Average Extent of Mobility Improvement was calculated by adding total numerical values of response (table 3) divided by the total number of animals responding to each compound.

note 2: Additional clinical studies on privately owned dogs exhibiting signs of locomotor dysfunction are in progress at veterinary hospitals throughout the United States. Further studies will be undertaken to determine if supplemental Ester-C ascorbate would be beneficial as a biological antioxidant or in the prevention of specific locomotor disorders, such as Canine I-Ep Dysplasia or Osteochrondritis.

BIBLIOGRAPHY

Adkins, T. O. and Kronfeld, D. S. : Diet of racing sled dogs affects erythrocyte depression by stress. *Canadian Vet. J.*,23:260-3, 1982.

Allen, T. A., and Hand, M. S. : Conditionally Essential Nutrients. Proc. 8th *ACVIM Forum*, pp. 809-811, 1990.

Anderson, R. : Ascorbic Acid and Immune Function. *In Vitamin Co Ascorbic Acid*- ed. by Counsell, I. N., and Hornig, D. H. ppo 249-272, Applied Science Publishers, London, 1981.

Belfield, W. O. :Chronic subclinical scurvy and canine hip dysplasia. *Veterinary Medicine/Small Animal Clinician*. 1399-1401, 1976

Berge, G. E. : Polyascorbate (C-Flex), an interesting alternative by problems in support and movement apparatus in dogs. *Norwegian Veterinary Journal*. 102:579-581, 1990.

Bielski, B. H. J.., Richter, H. W., and Chan, P. C. : Some properties of the ascorbate free radical. *Ann. N. Y. Acad. Sci.*, 258:231-237, 1975.

Block, G. : Vitamin C and cancer prevention: the epidemiological evidence. *Am. J. Clin. Nutr.*, 51270s-282s, 1991.

Bodannes, R. S., and Chan, P. C. : Ascorbic acid as a scavenger of singlet oxygen. *F. E. B. S. Letters*, 105: 195-196, 1979.

Bush, M. J., and Verlangieri, A. J. : An acute study on the relative gastrointestinal absorption of a novel form of calcium ascorbate. *Res. Commun. Chem. Path. Pharm.*, 57, 1987.

Dockter, C., unpublished data.

Donoghue, S., Kronfeld, D. S., and Banta, C. A. : A possible vitamin C requirements in racing sled dogs fed a high fat diet. In: *Nutrition, Malnutrition and Diet in Dogs and Cam* ed. by Meyer, H. and Kienzle, E. pp. II 0- 1 14, Hanover: Tierarztliche Hochschule, 198 8.

Fay, M. J., and Verlangieri. : Stimulatory action of calcium L-threonate on ascorbic acid uptake by a human T-lymphoma cell line. *Life Sciences*, AEO 13 77-13 81, 1991

Fay, M. J. : *Possible effects of ascorbic acid metabolites on the cellular uptake of ascorbic acid and other compounds. A dissertation for degree of Doctor of Philosophy*, University of Mississippi, 1992.

Goldschniidt, M. C. : Reduced bacteriocidal activity in neutrophils from scorbutic animals and the effect of ascorbic acid on these target bacteria in vivo and in vitro, *Am J. Clin. Nutr.*, 5. 4-1214s-1220s, 1991.

Goodman, S., *Vitamin C; The Master Nutrient*, Keats Publishing, Inc., Connecticut, 1991.

Harakeh, S.,, Jariwalla, R. J., and Pauling, L. : Supression of human immunodeficiency virus replication by ascorbate in chronically and acutely affected cells. Proc. Nati, Acad. Sci., n-7245-7249, 1990.

Hitt, M. E. : Superoxide radicals:what are they and why do we care! Proc. 8th *ACVIM Forum*, pp 777-780, 1990.

Horton, R. H., and Moran, L. A. : *Principles of Biochemistry*, Prentice-Hall, Inc., New Jersey, 1993.

Kozlenko, R. : New light on C - cellular absorption of vitamin C and protection against heart disease. pp 3 8-42. *New Editions Health World* 1994.

Kronfeld, D. S. : *Vitamin and Mineral Supplementation for Dogs and Cats-*. Veterinary Practice Publishing Company, California, 1989.

KronfeldD. S. :Stress supplements: Protein and vitamin C. *Purebred Dogs/Kennel Gazzette*, 100. 10:8-9, 1983.

Lewis, D. L., Morris, Jr. M. L., and Hand, M. S.,: *Small Animal Clinical Nutrition* Mark Morris Assoc., Kansas, 1990.

Newman, N. L., unpublished data.

Prinz, W., Bortz, R.., and Hersh, M. : The effect of ascorbic acid supplementation on some parameters of the human immunological defense system. Int. J. *Vit Nutr. Res.*47:248-257, 1977.

Retsky, K. L., Freeman, M. W., and Frei, F. : Ascorbic acid oxidation products protect human LDL against atherogenic mortification rather than pro-oxidant activity of vitaniin C in the presense of transition metal ions. *J. Biol. Chem.* 26a, No. 2: 1404-1309, 1993.

Selvidge, L. A., and Verlangieri, A. J. : Inhibition of arylsulfatase b by ascorbic acid. *Res. Commun. Chem. Path. Pharmacol.*, 7_3-253-256, 1991.

Simon, J. A. : Vitamin C and cardiovascular disease: a review. *J. Am. Coll. Nutr.*, 73-253-256, 1991.

Smith, C. : More on vitamin C. *Natural Pet.* Vol 2, No. 6:18-19, 1993.

Stein, D. : *Natural Healing for Dogs and Cat*, The Crossing Press, California, 1993.

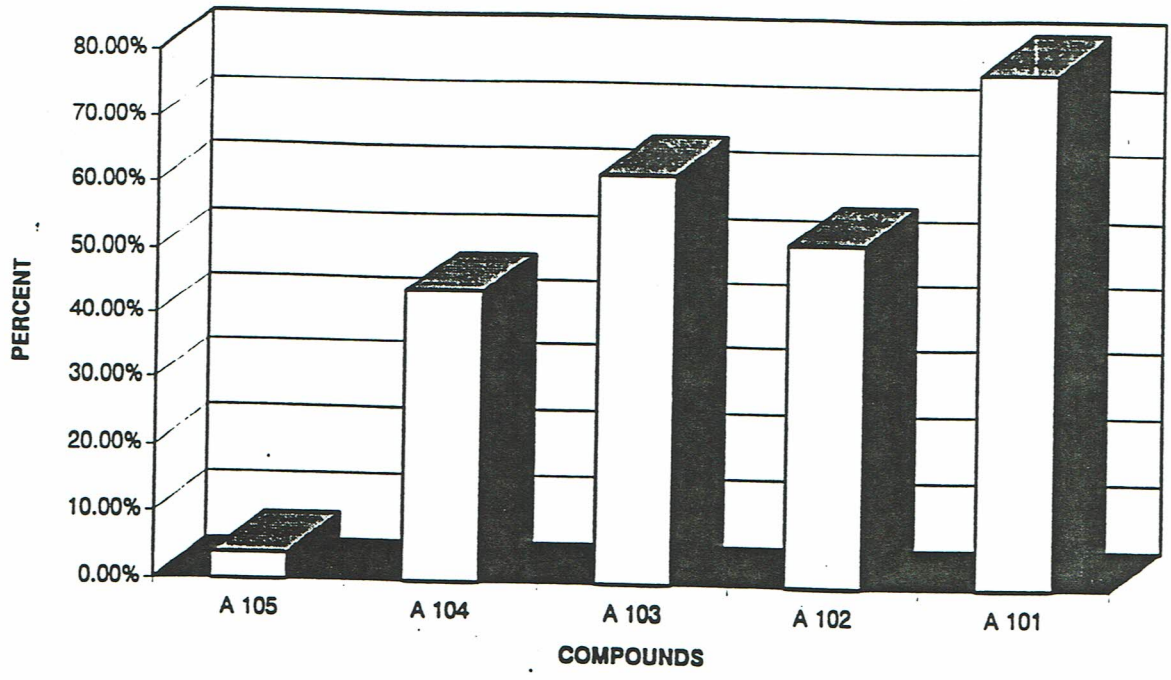
Verlangieri, A. J., Fay, M. J., and Bannon, A. W. : Comparison of the anti-scorbutic activity of L-Ascorbic acid and Ester-C in the non-ascorbate synthesizing Osteogenic Disorder Shionogi (ODS) rat. *Life Scie~ces*, 48:2275-2281, 1991.

# TABLE 4A

| GROUP I | Comp. 101 | | Comp. 105 | | Comp. 101 |
|---|---|---|---|---|---|
| CLEO | 1 | ↔ | 0 | ↔ | 2 |
| FIONA | 2 | ↓ | 0 | ↔ | 2 |
| TESS | 2 | ↓ | 0 | ↔ | 2 |
| BEAU | *a | N/A | N/A | N/A | N/A |
| COLLEEN | *b | N/A | N/A | N/A | N/A |
| BRODY | 1 | ↔ | 0 | ↔ | 3 |
| SUZIE | 3 | ↓ | 0 | ↔ | 2 |
| TEDDY | 3 | ↓ | 0 | ↓ | 3 |
| MURPHY | 0 | ↔ | 0 | ↔ | 1 |
| **GROUP II** | **Comp. 102** | | **Comp. 104** | | **Comp. 101** |
| KUMI | 2 | ↓ | 3 | ↓ | 3 |
| SHADOW | 2 | ↔ | 3 | ↓ | 3 |
| HARLEY | 1 | ← | 1 | ↔ | 2 |
| MEGAS | 2 | ← | 2 | ↔ | 3 |
| CINDERELLA | 0 | ↔ | 1 | ↓ | 1 |
| BUSTER | 0 | ↔ | 2 | ↓ | 0 |
| COSMO | 0 | ↔ | 0 | ↓ | 1 |
| WANDA | 1 | ↔ | 0 | ↔ | 1 |
| SUZIE | 0 | ↔ | 0 | ↔ | 2 |
| **GROUP III** | **Comp. 103** | | **Comp. 101** | | **Comp. 104** |
| ARROW | 2 | ↓ | 2 | ↓ | 1 |
| VICTOR | 0 | ↔ | 1 | ↔ | 0 |
| CURRY | 1 | ↔ | 2 | ↓ | 1 |
| HONEY | 1 | ↔ | 3 | ↓ | 0 |
| REX | 2 | ↓ | 0 | ↔ | 0 |
| COYOTE(repl. Pebbles) | N/A | ↔ | 0 | ↔ | 0 |
| PEBBLES | 0 | ↔ | 1 | ↔ | 0 |
| GARTH | 1 | ↔ | 0 | ↔ | 0 |
| ROSEY | 0 | ↔ | 0 | ↔ | 0 |
| **GROUP IV** | **Comp. 104** | | **Comp. 102** | | **Comp. 101** |
| SPECTOR | 0 | ↔ | 0 | ↔ | 1 |
| BRUNHILDA | 0 | ↔ | 0 | ↔ | 0 |
| AMY | 1 | | 2 | ↓ | 2 |
| SALLY | 0 | ↔ | 0 | ↔ | 1 |
| CHEWBACCA | 0 | ↔ | 0 | ↔ | 0 |
| MAXINE | 1 | ↔ | 3 | ↔ | 3 |
| LUCKY *e | 0 | | 2 (101) | ↓ | 3 |
| TAHOE | 1 | ↓ | 2 | ↓ | 3 |
| TIPPY | 1 | ↓ | 3 | ↓ | 3 |
| **GROUP V** | **Comp. 105** | | **Comp. 101** | | **Comp. 105** |
| SURGE | 0 | ↔ | 0 | ↔ | 0 |
| TOOTLES | 0 | ↔ | 1 | ↔ | 0 |
| MELVA | 0 | ↔ | 1 | ↓ | 0 |
| RAOUL | 1 | ↔ | 3 | ↓ | 3 |
| GEORGINA | 0 | ↔ | 1 | ↔ | 0 |
| SMOOCHIE | 0 | ↔ | 0 | ↔ | 0 |
| GEORGE | 1 | ↔ | 1 | ↓ | 0 |
| CLAIRE | 0 | ↔ | 0 | ↔ | 0 |
| MAGGIE | N/A | N/A | 2 | ↓ | 2 |

TABLE 4B

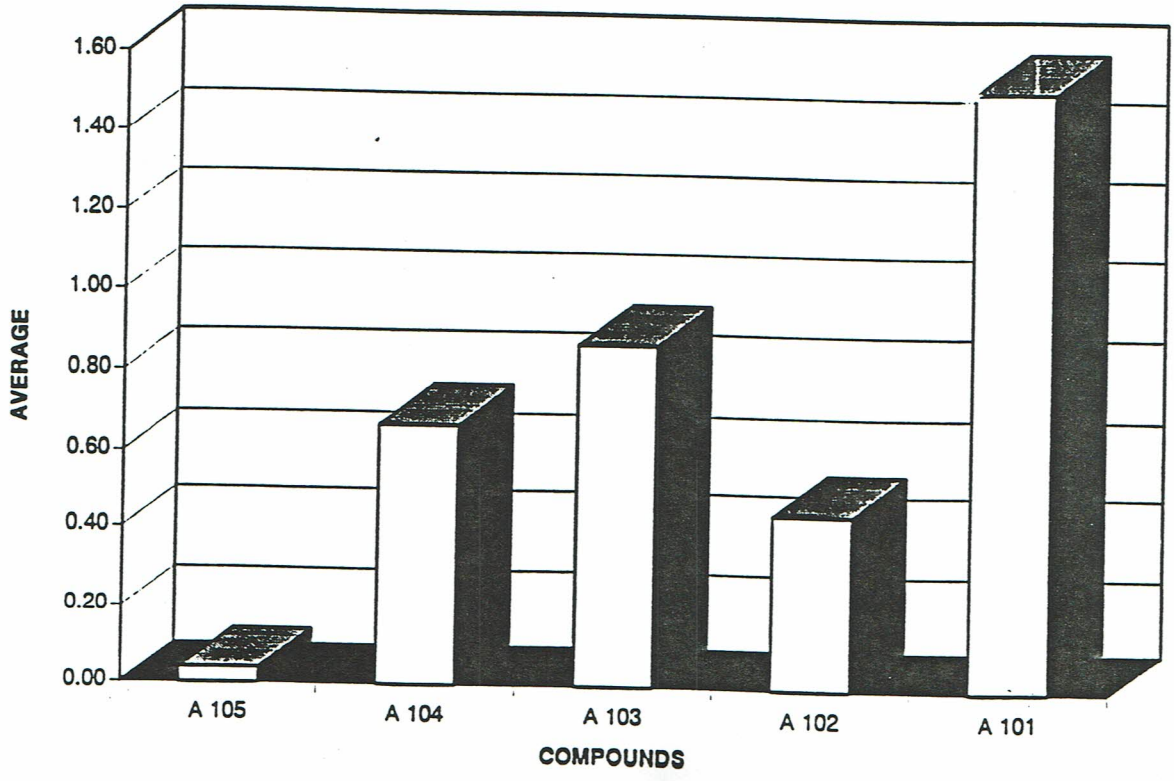
PERCENT OF CASE STUDIES SHOWING IMPROVEMENT




| Percentage Mobility Improvement Score | A 105 | A 104 | A 103 | A 102 | A 101 |
|---|---|---|---|---|---|
| | 4.00% | 44.00% | 62.00% | 52.00% | 78.00% |

TABLE 4C

AVERAGE MOBILITY IMPROVEMENT SCORE ( AMIS )




| Average Mobility Improvement Score | A 105 | A 104 | A 103 | A 102 | A 101 |
|---|---|---|---|---|---|
| | 0.04 | 0.67 | 0.87 | 0.45 | 1.52 |

Photograph: Don Douglas

# VITAMIN C

## New Forms and New Uses in Dogs

**The common wisdom is that humans, other primates, guinea pigs, and a few birds need to get vitamin C in their diets. Since the rest of the animals produce it within their own bodies, they no doubt make enough, right? Well, maybe not. In this article, a doctor of veterinary medicine looks at the effects of vitamin C supplementation in dogs and its benefits in fighting arthritis, and other ailments.**

*Dr. Brown is a small animal veterinarian and consultant to several companies that manufacture and market natural pet products. He can be contacted at P.O. Box 1177, St. Augustine, FL 32085.*

**L. Phillips Brown, D.V.M.**

Dogs of all ages suffer with various joint and spinal disorders, including hip dysplasia, osteoarthritis, non-specific arthritis, osteochrondritis, spondylitis and spondylosis. Treatment often consists of rest, surgery and/or steroids, nonspecific anti-inflammatory drugs (NSAIDS), aspirin, penicillamine or methotrexate. Many therapeutic compounds produce only short-term benefits and may actually accelerate the progression of joint destruction.

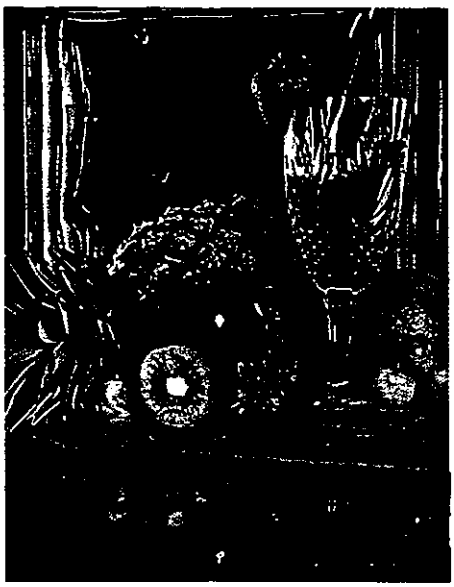
### Nutritional Considerations

Vitamin C is a vital nutrient in bone and cartilage metabolism. Although dogs, unlike humans, can manufacture their own vitamin C, they may not produce enough to counter the effects of aging, stress, inherited dysfunctions, environmental irritants and poor quality or high fat pet foods.

In fact, early studies in dogs and horses suggest that daily vitamin C supplementation might be beneficial in reducing chronic inflammation. Unfortunately, ordinary Vitamin C may cause gastrointestinal upsets in dogs. A form of vitamin C that would promote higher levels of intracellular ascorbic acid without negative side effects would be a useful and unique product.

## Clinical Study

The effects of different forms of vitamin C on various locomotor dysfunctions of dogs were investigated by veterinarians at The Best Friends Animal Sanctuary in Utah. Over 100 case studies were evaluated, using varying strengths and combinations of mineral ascorbates, ascorbic acid and microcrystalline cellulose during a six- month period. The results indicate that a patented vitamin C ascorbate/vitamin C metabolite complex, administered orally, may have application for the reduction of discomfort associated with nonspecific, chronic inflammatory disorders of dogs. The vitamin C ascorbate/metabolite complex used in the study was Ester-C®.



## Product Background

According to the manufacturer, Inter-Cal Corporation, of Prescott, Arizona, Ester-C is a patented ascorbate supplement containing calcium ascorbate, naturally occurring dehydroascorbate and the vitamin C metabolite, threonate. Threonate permits ascorbate to be more rapidly absorbed from the gastrointestinal tract, to cross cell membranes more effficiently, reach higher cellular levels and be excreted more slowly than ordinary vitamin C. Ester-C ascorbate is pH neutral and believed to be water and partially fat soluble.

## Mechanism Of Action

Vitamin C may act as an immunoresponsive and chrondrogenerative agent. In degenerative (i.e., aging) or inflammatory conditions, collagen breakdown is excessive, resulting in joint discomfort and skeletal changes. A product that would provide high and prolonged levels of ascorbic acid could help increase the compression resistance of cartilage, mobilize white blood cells to the site of inflammation, and enhance prostaglandin synthesis. The net result would be increased comfort and mobility.

## Conclusion

**Seventy-eight percent of the study dogs receiving Ester-C calcium ascorbate showed improved mobility within four to five days.**

The consistency and degree of response demonstrated that Ester-C calcium ascorbate provided symptomatic relief to the study dogs suffering from selected chronic joint and musculoskeletal disorders. The findings that the condition of many of the "improved" dogs deteriorated rapidly upon discontinuance of Ester-C ascorbate further verified its usefulness as primary or adjunctive therapy. These studies reinforced the earlier observations that supplemental Ester-C calcium ascorbate corrected mobility problems of dogs and horses.

The findings suggest that, although dogs can manufacture endogenous ascorbic acid, the amount produced may not be sufficient to prevent or counteract stresses associated with aging, injury or joint malpositioning.

**From the results of this study, there is significant evidence to recommend oral Ester-C calcium ascorbate in the management of non-specific musculoskeletal disorders of dogs.**

## References


Adkins, T.O. and Kronfield, D.S.: Diet of racing sled dogs affects erythrocyte depression by stress. *Canadian Vet. J.*, 23:260-3, 1982

Allen, T.A., and Hand, M.S.: Conditionally Essential Nutrients. *Proc. 8th ACVIM Forum*, pp. 809-811, 1990

Belfield, W.O.:Chronic subclinical scurvy and canine hip dysplasia. *Veterinary Medicine/Small Animal Clinician.* 1399-1401, 1976

Berge, G.E. : Polyascorbate (C-Flex), an interesting alternative by problems in support and movement apparatus in dogs. *Norwegian Veterinary Journal* 102:579-581, 1990.

Dockter, C unpublished data.

Donoghue, S., Kronfeld, D.S. and Banta, C.A.: A possible vitamin C requirements in racing sled dogs fed a high fat diet, In: *Nutrition Malnutrition and Diet in Dogs and Cats* ed. by Meyer, H. and Kienzle, E. pp. 110-114, Hanover: Tierarztliche Hochschule, 1988.

Fay, M.J.: Possible effects of ascorbic acid metabolites on the cellular uptake of ascorbic acid and other compounds. A dissertation for degree of Doctor of Philosophy, University of Mississippi, 1992.

Goodman, S., Vitamin C: *The Master Nutrient*, Keats Publishing, Inc. Connecticut, 1991.

Kronfeld D.S.: Stress supplements: Protein and vitamin C. *Purebred Dogs/ Kennel Gazette*, 100. 10:8-9. 1983.

Lewis, D.L., Morris, Jr. M.L., and Hand, M.S.,: *Small Animal Clinical Nutrition* Mark Morris Assoc., Kansas, 1990.

Newman, N.L. unpublished data.

Smith, C.: Moro on vitamin C. *Natural Pet.* Vol 2, No. 6:18-19. 1993.

Stein, D.: *Natural Healing for Dogs and Cats*, The Crossing Press, California, 1993.

# Equine Degenerative Joint Disease

## *A Nutritional Approach with Ester-C®*

by N. Lee Newman, DVM

*Once agile and sweet-tempered, Mischief, a 5 year old Morgan gelding, turned sour, bared teeth to horse and human alike and became progressively lame. Prescribed pain killers and anti-inflammatory drugs provided only minimal relief.*

> ***"...over 90% of the horses had shown good to excellent response to Ester-C® calcium ascorbate."***

*The new owner of the Anglo-Arab gelding looked forward to successful competition in dressage. But soon his once fluid movements were replaced by an awkward, stilted gait. Within a few months the gelding had become increasingly lame and by two years could only be ridden under heavy doses of phenylbutezone.*

Both horses suffered from Degenerative Joint Disease (DJD), the most common crippler of performance horses. DJD can affect any horse at any age and is usually progressive.Physitis,osteochondrosis dissecans, traumatic and infective degenerative arthritis, ringbone, bone spavin and knee, fetlock and stifle arthritis and inflammation are all forms of DJD. The economic effect of any one of these conditions in a well trained, conditioned performance horse, as well as the emotional trauma of watching a well-loved companion animal suffer, is devastating.

Although some horses remain useful for light riding, serious athletic endeavors such as racing, jumping, reining, endurance or dressage are impossible. Therapy is limited to pain suppression or surgery to prevent joint movement. The drugs used to combat pain and inflammation are expensive and must be continued long-term, often with undesirable side effects. Other modalities, such as acupuncture, cold laser and magnetic field therapy, have been used with some success. Nutritional supplements, such as yucca, glycosaminoglycans, chondroitin sulfate, hyaluronic acid, methylsulfonylmethane and dimethylglycine have provided mixed results. More and more veterinary practitioners are now advocating the use of vitamin C (ascorbic acid).

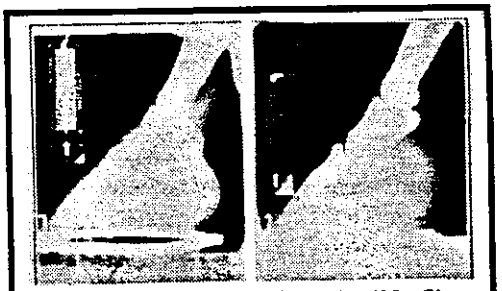


Fig. 1 June '91– Cherry Queen periarticular ringbone at P2-P3 pastern joint

Fig. 2 Jan '92– Cherry Queen reported to be 100% sound after Ester-C® therapy.

Vitamin C is involved in hundreds of basic biochemical functions. Specifically, it may act as a chondro-regenerative agent to restore and maintain the integrity of collagen, connective tissue and bone. Vitamin C may be an immuno-responsive agent, acting to mobilize white blood cells and decrease synthesis of prostaglandins. Furthermore, this important vitamin serves as a scavenger of free radicals (unpaired electrons) which may damage the integrity of cell membranes. But does supplemental vitamin C have a place on the equine practitioner's shelf? As evidence of the benefits mount, veterinary clinicians are saying, "yes".

Horses, unlike humans, manufacture endogenous ascorbic acid by way of internal enzymatic processes. However, the amount produced may not be sufficient to counter the effects of age, husbandry, condition and work. Athletic performance could easily use up the body's supply or exaggerate the physiological requirement for this vitamin. The problem with oral supplementation of

ordinary ascorbic acid is that it often causes gastrointestinal distress, has a bitter, astringent taste and is rapidly excreted from the body before it can be transported to needy tissue.

Veterinarians have recently become interested in a patented ascorbate supplement, Ester-C®, that contains calcium ascorbate, naturally occurring dehydroascorbate and the vitamin C metabolite, threonate. According to the manufacturer, Inter-Cal Corporation of Prescott, Arizona, threonate permits ascorbate to be more rapidly absorbed from the gastrointestinal tract, cross cell membranes more efficiently, reach higher intracellular levels and be excreted more slowly than ordinary vitamin c.

In 1992, clinical trials to assess and quantify the effects of Ester-C® calcium ascorbate on DJD in horses were begun. Over an 18 month period, horses diagnosed with a variety of DJD conditions exhibiting moderate to severe lameness were given oral Ester-C®. Preliminary radiographs with veterinary examinations classified the severity of lameness and degree of response in accordance with the American Association of Equine Practitioners Lameness Classification Scores.

At the conclusion of the 18 month trial period, over 90% of the horses had shown good to excellent response to Ester-C® calcium ascorbate. 80% of the "improved" horses remained sound and usable even after the supplement was discontinued. Ongoing clinical trials are currently being conducted to monitor long term effects.

Up to now, therapeutic agents giving temporary relief and/or surgery were treatments of choice for horses with Degenerative Joint Disease. Current studies are demonstrating the importance of alternative modalities and therapeutics. From our own experience, it seems that Ester-C® calcium ascorbate may provide yet another alternative.

Anyone interested in further information or participating in these clinical trials may contact:

Dr. N. Lee Newman,
P. O. Box 255,
Middletown,VA 22645
Fax: (703) 869-0979

## Respiratory Study Subjects Needed

*Chronic allergic respiratory disease, or "heaves", occurs in 5-10% of all horses. Thought to be triggered by seasonal inhalant allergens, this condition can be quite debilitating. It is usually treated with antihistamines or bronchodilators, unless the heavy mucous accumulation leads to a secondary infection.*

*During the evaluation of the effects of Ester-C® calcium ascorbate on DJD in horses, it was noted that several of the horses with a past history of heaves remained symptom free. One stallion who was no longer receiving Ester-C® developed a cough at the height of the grass pollen season. The cough subsided when Ester-C® was again administered.*

*Dr. Newman is seeking horses with documented chronic allergic pulmonary disorder to evaluate the role Ester-C® calcium ascorbate both as a nutritional supplement and a respiratory support agent. Please contact her at the address which follows.*

**References**


- Stashak, Ted S., *Adam' Lameness in Horses*, 4th Edition, Lea & Febiger, 1987, pp. 486-786.
- Kohnke, John, *Feeding and Nutrition*, Birubi Pacific, 1992,p. 181.
- Goodman, Sandra, *Vitamin C: The Master Nutrient*, Keats Publishing, 1991.
- Ralston, Sarah, *Clinical Nutrition of Adult Horses*, The Veterinary Clinics of North America, Vol. 6, No.2, W.B. Saunders Co., August, 1990, p. 345.
- Verlangieri, Anthony J., in Ester-C Research Summary, Inter-Cal Corp., Prescott, AZ.,1987-1991.
- Guideline for Veterinary Service and Judging of Equestrial Events, 4th ed., American Association of Equine Practitioners, Lexington, KY., 1991, p. 19.

VOL. 57, NO. 1
JULY 1987

Research Communications in
Chemical Pathology and Pharmacology

# AN ACUTE STUDY ON THE RELATIVE GASTRO-INTESTINAL ABSORPTION OF A NOVEL FORM OF CALCIUM ASCORBATE

Marilyn J. Bush and Anthony J. Verlangieri[1]

Atherosclerosis Research Laboratories,
Department of Pharmacology, School of Pharmacy,
University of Mississippi, University, MS 38677

ABSTRACT. Several functions of L-ascorbic acid (vitamin C) have been suggested in addition to its role in the prevention of scurvy. Consequently, a controversy has arisen over the daily intake of the vitamin which will afford maximum benefits. Rapid cellular uptake and delayed renal excretion of ascorbic acid would be conducive to providing optimum cellular concentration for biochemical activity. ESTER-C® (patent pending), a complex consisting of L-ascorbic acid and $Ca^{++}$, has been recently developed by Inter-Cal Corporation (421 Miller Road, Prescott, AZ 86301). It has been proposed that the structure of ESTER-C® may render it more readily absorbed and less rapidly excreted than the acid or salt form of the vitamin. To test this hypothesis, ESTER-C® and L-ascorbic acid were administered to two groups of rats. Blood was sampled at 20, 40, 80, 160 and 240 minutes and plasma analyzed for ascorbic acid. As urine appeared in collection cups, it was tested qualitatively for the presence of ascorbic acid. The plasma concentration of ascorbic acid was higher in ESTER-C® treated rats at 20, 40 and 80 minutes than in rats given L-ascorbic acid. Ascorbic acid was detected in the urine of animals administered ESTER-C® later than in those treated with L-ascorbic acid. These results support the hypothesis that ESTER-C® is absorbed more readily and excreted less rapidly than L-ascorbic acid.

INTRODUCTION. Besides its established anti-scorbutic function, ascorbic acid (vitamin C) is involved in many redox biosynthetic pathways, as in the formation of hydroxyproline and hydroxylysine during the synthesis of procollagen on the endoplasmic reticulum of connective tissue cells (5). Ascorbic acid may also serve in connective tissue metabolism as a carrier of activated sulfate needed for the synthesis of glycosaminoglycans (GAGs) (7), as well as an inhibitor of aryl sulfatase B, which desulfates GAGs (8). Vitamin C has been implicated in immune system function (1,6), cholesterol metabolism (4), cardiovascular disease (9) and cancer treatment and/or prevention (3,10). Rapid cellular uptake of vitamin C, along with delayed renal excretion may promote optimal biochemical activity. Inter-Cal Corporation (421 Miller Road, Prescott, AZ 86301) has recently developed a product, ESTER-C® (patent pending), which may

[1]Author to whom reprint requests should be addressed.

be a $Ca^{++}$-chelate of ascorbic acid. Although the exact structure has not been elucidated, it is expected to be less ionized than L-ascorbic acid, and therefore more lipid soluble. Preliminary research indicates that the molecular weight of ESTER-C® may be higher than that of L-ascorbic acid, possibly rendering it less likely to be rapidly filtered by the kidney. Theoretically, the net result would be to attain a higher plasma concentration of ascorbic acid compared to the acid or salt form of L-ascorbic acid.

To test this hypothesis, both compounds were administered acutely to rats, and plasma concentrations of ascorbic acid at five time intervals over a period of 240 minutes were ascertained, as well as time of detection of ascorbic acid in the urine of these animals.

MATERIALS AND METHODS. Twelve Charles-River male Spraque-Dawley albino rats weighing 300-400 grams were individually caged and acclimated for 7 days prior to the beginning of the study. At the end of the acclimation period, the rats were randomly assigned to one of three groups. After a 12-hour fast, the animals were treated as follows. Group I, n=5, received 20 mg/kg body weight L-ascorbic acid in distilled water by gavage. Similarily, group II (n=5) was administered 27.4 mg/kg body weight ESTER-C® (dose corrected for $Ca^{++}$ concentration) in the same manner. Group III (n=2) served as a negative control and provided normal baseline data, receiving only distilled water. After dosing, the animals were placed in metabolic cages (Nalgene, Rochester, NY). Urine was collected as it appeared and ascorbic acid was measured qualitatively using Uralyte Fast Tabs (Genus Diagnostics, 1800 Pershing Road, Decatur, IL 62526). Relative positive excretion (5 mg% and 10 mg%) and time of collection was recorded for each animal. Blood samples were taken by sinus orbital puncture under light ether anesthesia at 20, 40, 80, 160 and 240 minutes posttreatment. Whole blood was placed in 3 ml test tubes containing 4.5 mg EDTA and centrifuged immediately for 5 minutes. Five-hundred µl of plasma was removed and placed in 25 µl of perchloric acid to stabilize the ascorbic acid. Plasma samples were then frozen for later analysis of ascorbic acid by HPLC (2). Results were calculated and expressed as µg ascorbic acid/ml plasma.

All statistics were calculated via Statistical Analysis System (SAS Institute, Inc., Box 8000, Cary, NC 27511) software. The ANOVA (repeated measures model) procedure was used to determine differences in plasma ascorbic acid concentration between groups I and II, while the one-way ANOVA was used to ascertain differences in time of appearance of ascorbic acid in the urine of animals in these two groups.

RESULTS. Table I displays the mean (± standard deviation) plasma ascorbic acid concentration for the treatment groups at each sample collection time in µg/ml. Figure 1 presents these data as linear regression plots of concentration versus time over the first 80 minutes of sampling, yielding an absorption rate of 0.099 µg/min for ESTER-C® and 0.026 µg/min for L-ascorbic acid. It is apparent from Table 1 that ESTER-C® absorption was greater than that of L-ascorbic acid at 20, 40 and 80 minutes. At 160 minutes, the absorption rate of both compounds converges, with that of L-ascorbic

acid surpassing ESTER-C® at 240 minutes. Analysis of variance using a repeated measures model confirmed the significance ($p=0.0001$) of higher plasma ascorbic acid concentrations in the ESTER-C® treated animals over time.

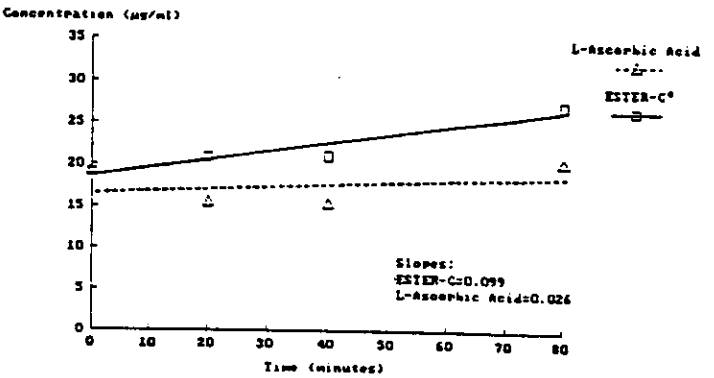
TABLE I
PLASMA ASCORBIC ACID CONCENTRATION IN RATS
TREATED WITH L-ASCORBIC ACID OR ESTER-C®

| Time (minutes) | Ascorbic Acid (µg/ml) | ESTER-C® (µg/ml) |
|---|---|---|
| 20 | 15.42 ± 8.34[a] | 20.96 ± 2.77 |
| 40 | 15.28 ± 9.17 | 21.02 ± 9.04 |
| 80 | 20.36 ± 8.23 | 27.24 ± 6.79 |
| 160 | 24.24 ± 8.24 | 25.38 ± 4.36 |
| 240 | 32.48 ± 5.71 | 28.36 ± 2.32 |

[a]Mean ± Standard Deviation

FIGURE 1

Ascorbic Acid Concentration vs Time
in Rats Treated with
L-Ascorbic Acid or ESTER-C®




Linear Regression Derivation
of Absorption Rates

Average time (± S.D.) of initial appearance of ascorbic acid in the urine was 103.25 (± 11.81) minutes post-treatment for the L-ascorbic acid treated animals, and 208.00 (± 32.51) minutes for animals dosed with ESTER-C®. Evaluation of the urine and plasma ascorbic acid data indicates that ESTER-C® (as ascorbic acid) is excreted much less rapidly than L-ascorbic acid, in spite of higher plasma levels. At 103 minutes, plasma concentration of ascorbic acid was approximately 19 µg/ml in the L-ascorbic acid group. For the ESTER-C® group, this concentration was 26 µg/ml at 208 minutes. According to one-way ANOVA, the appearance of ascorbic acid in the urine of the ESTER-C® animals was significantly later than in the L-ascorbic acid group.

DISCUSSION. The results of this investigation suggest that ESTER-C® is absorbed more readily than L-ascorbic acid, thus supporting the theory that it has a less ionic character, is more lipid soluble, and passes the mucosal barriers more rapidly than L-ascorbic acid. At 160 minutes posttreatment, plasma concentration of ascorbic acid in the ESTER-C® treated animals begins to stabilize. This could be a consequence of vitamin distribution throughout the body, exceeding the renal threshold, or reduction of blood volume by repeated bleeding of the animals.

Ascorbic acid was detected in the urine of L-ascorbic acid treated animals twice as early as that of those treated with ESTER-C®. Less rapid excretion of ESTER-C® compared with L-ascorbic acid, concurrent with higher plasma values of ascorbic acid, is consistent with a larger molecular weight and/or size of ESTER-C® favoring slower renal filtration and subsequent excretion.

If the unique structure of ESTER-C® promotes more rapid absorption and delayed excretion of ascorbic acid compared to the salt or acid forms of the vitamin, as the results of this experiment clearly imply, it may be advantageous in increasing circulating ascorbic acid concentration and promoting rapid tissue saturation. Such a result could be significant in reducing morbidity and mortality due to cardiovascular disease (9), cancer (3,10), and respiratory illness (1,6), as well as other disorders in which relative ascorbic acid deficiency has been implicated.

Further investigation of ESTER-C® is required to elucidate its structure, its cellular transport mechanism, and its bioactivity.

The authors would like to express their appreciation to Anthony Bannon for his technical assistance, and to Diane Williams for her expert typing of the manuscript.

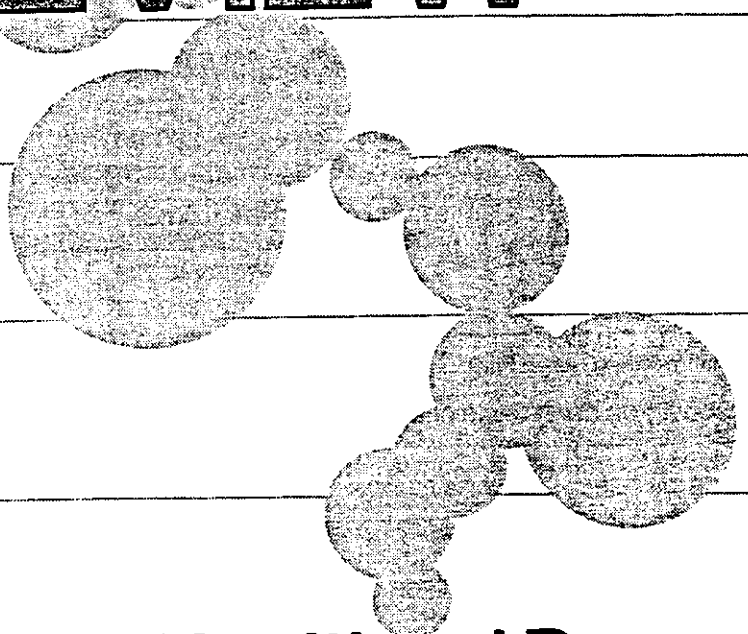
## REFERENCES

1. Anderson, R. (1981). In Counsell, JN, Hornig, DH (eds). Vitamin C: ascorbic acid. Applied Science Publishers, London p. 249-272.
2. Baker, J.K., Kapeghian, J.C., and Verlangieri, A.J. (1983). J. Liquid Chrom. 6, 1319-1332.
3. Cameron, E. and Pauling, L. (1976). Proc. Natl. Acad. Sci. USA 73, 3685-3689.
4. Ginter, E. (1973). Science 179, 702-704.
5. Levine, M. (1986). N. Engl. J. Med. 314, 892-902.
6. Prinz, W., Bortz, R., Bregin, B., and Hersch, M. (1977). Int. J. Vitam. Nutr. Res. 47, 248-257.
7. Verlangieri, A.J. and Stevens, J.W. (1979). Blood Vessels 16, 177-185.
8. Verlangieri, A.J. and Selvidge, L.A. (1987). Fed. Proc. 46 No. 3, 415.
9. Verlangieri, A.J., Kapeghian, J.C., El-Dean, S., and Bush, M.J. (1985) Medical Hypothesis 16, 7-15.
10. Wittes, R.E. (1986). Vitamin C and cancer. N. Engl. J. Med. 312, 178-179.

Copyright © 1987 By
PJD Publications Ltd., Box 966, Westbury, N.Y. 11590 USA

Volume 10, Number 1 – First Quarter
January, 1990

# INTERNATIONAL CLINICAL NUTRITION REVIEW



## Recent Nutritional Research with Potential Clinical Application

INTEGRATED THERAPIES PTY. LIMITED
SYDNEY — AUSTRALIA

## BIOAVAILABILITY STUDIES

# Comparative Studies of "ESTER C" Versus L-Ascorbic Acid

Jonathan V. Wright M.D.
(Tahoma Clinic)

Raymond M. Suen M.T., ASCP
Floyd R. Kirk
(Meridian Valley Clinical Laboratories)

24030 132nd Avenue S.E.
Kent, Washington 98042 U.S.A.
Fax: 206 631 8691

The manufacturers of a novel form of ascorbate ("Ester C") asked us to carry out studies comparing aspects of its absorption, distribution, retention and excretion with L-ascorbic acid. Details of these studies follow, which show better retention, higher WBC levels, less excretion and less associated oxalate production for the "Ester C" form.

According to its manufacturer, "Ester C" is "a complex mixture of many forms of vitamin C, including ascorbate, dehydroascorbate, and metabolites of vitamin C and calcium". The product is proprietary. We have no interest in it (financial or otherwise) besides the design, accurate completion and reporting of the details of this study, so we have not investigated the manufacturer's claims concerning the exact molecular nature of "Ester C".

For purposes of this study, we have accepted the manufacturer's claim that 4000mg "Ester C" contains 3000 mg ascorbate "with the rest being other metabolites of vitamin C and calcium". The results obtained appear to support this claim, as they indicate that we are indeed dealing with something other than a simple difference in quantity of ascorbate administered.

Parameters measured included serum ascorbate, WBC ascorbate, 24 hour urinary ascorbate and 24 hour urinary oxalate.

### METHODS, MATERIALS

Ascorbate was measured by methods standard to clinical chemistry [1,2]. Oxalate was measured with Sigma Chemical Kits catalog 590-10, 590-1, 590-4, 590-5, 0 6502, 0 6627. Instrumentation included B&L spectrophotometer 21.

Twelve men ages 27 to 45 were studied. All were on a diet excluding citrus, green leafy vegetables, and vitamin supplementation for one week prior to the start of the study.

After this week, and an overnight fast, blood was drawn. Serum and WBC ascorbate levels were determined. 24-hour urine specimens were collected and analysed for ascorbate and oxalate.

Copyright © 1990 Integrated Therapies

The 12 men were then divided into three groups of 4. While continuing the same diet, the groups took:

A. "Ester-C" 4000 mg. daily;
B. L-ascorbic acid 3000 mg. daily;
C. Citric acid 3000 mg. daily.

After ingestion of the above, blood specimens were taken at 0, 4, 8 and 24 hours; serum and WBC ascorbate were determined. Twenty-four hour urine specimen collections were made, and analysed for ascorbate and oxalate. After a two to six day (depending on job situation of participants) "washout" period, groups were switched as follows:

| | | | | | |
|---|---|---|---|---|---|
| "Ester-C" | → | Citrate | (Group A | → | Group C) |
| Ascorbate | → | "Ester-C" | (Group B | → | Group A) |
| Citrate | → | Ascorbate | (Group C | → | Group B) |

The same level of supplementation was taken by each group, and blood and urine specimen collections were made at exactly the same intervals as before, and again analysed for serum and WBC ascorbate, and 24-hour urinary ascorbate and oxalate.

Combined data from these specimen collections noted above are displayed in Figures 1 through 8.

The second ascorbate and "Ester C" groups continued taking these supplements at the same levels for 7 to 10 days. At the end of these periods, fasting blood specimens and 24-hour urine collections were taken. Data are displayed in Figures 9 through 12.

## RESULTS AND OBSERVATIONS

"Ester C" produced significantly higher serum levels than L-ascorbic acid at 4, 8 and 24 hours after ingestion, as well as after 7-10 days of continuous ingestion.

"Ester C" produced significantly higher WBC levels then L-ascorbic acid at 8 and 24 hours after ingestion, and after 7-10 days of continuous ingestion. WBC levels at 4 hours after ingestion were slightly higher in the "Ester C" group.

"Ester C" was associated with significantly and substantially less oxalate excretion in the first 24 hours after ingestion, as well as significantly less oxalate excretion after 7-10 days of continuous ingestion.

"Ester C" was associated with significantly and substantially less urinary ascorbate loss in both the first 24 hours of ingestion, and after 7-10 days of continuous ingestion.

## CONCLUSIONS

In this study, "Ester C" was clearly superior to L-ascorbic acid for serum and WBC levels produced, both short and intermediate term. Much less oxalate was excreted with "Ester C" administration than with L-ascorbic acid; this phenomenon is presumably associated with the higher serum and WBC levels found in the "Ester C" group.

Although this is only one study, its conclusions make a very strong suggestion that (aside from price considerations) "Ester C" is preferable to L-ascorbic acid for both short

and intermediate term supplementation. Not only is more ascorbate retained, and higher tissue levels achieved, but substantially less oxalate is excreted, lowering the risk of oxalate stone.

## REFERENCES


1. Henry RJ, Cannon DD, Winklemen JW, eds. Clinical Chemistry, Harper and Row, 1393-1398 (1974).

2. Seligson D, ed. Standard Methods of Clinical Chemistry, Academic Press, Vol.3, 35 (1961).

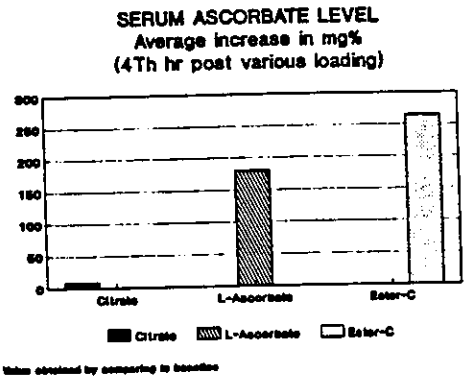




*Figure 1.*

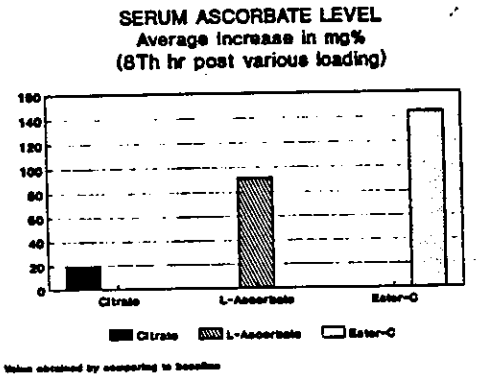



*Figure 2.*

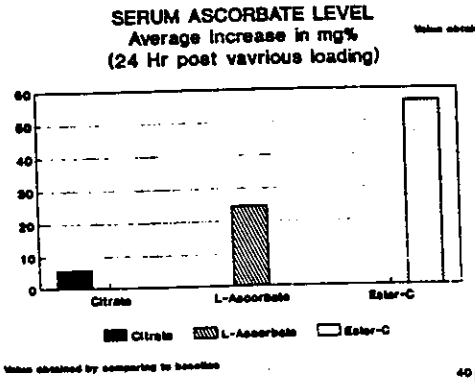



*Figure 3.*

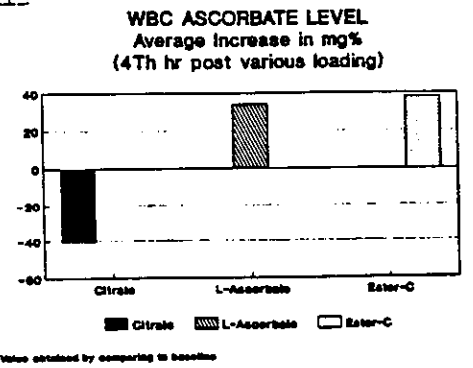



*Figure 4.*

Copyright © 1990 Integrated Therapies

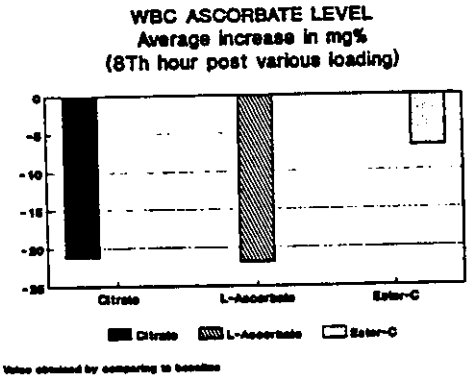



Figure 5.

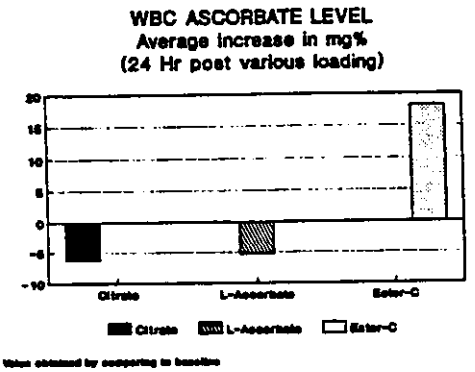



Figure 6.

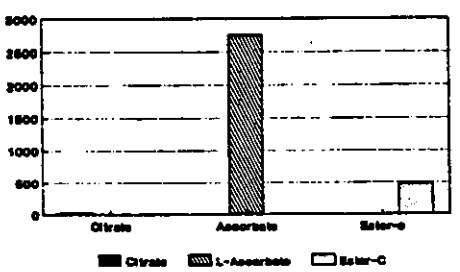



Figure 7.

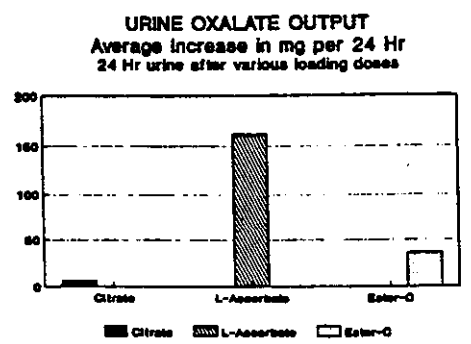



Figure 8.

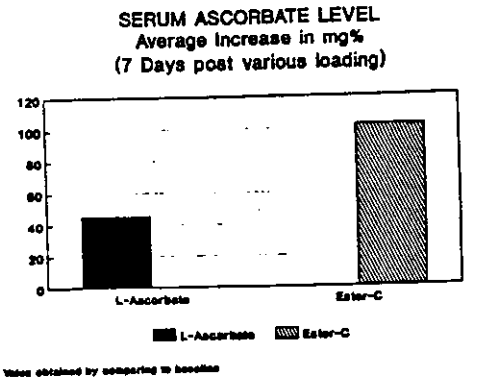



Figure 9.

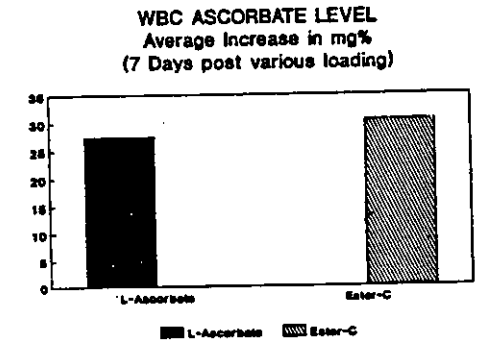



Figure 10.

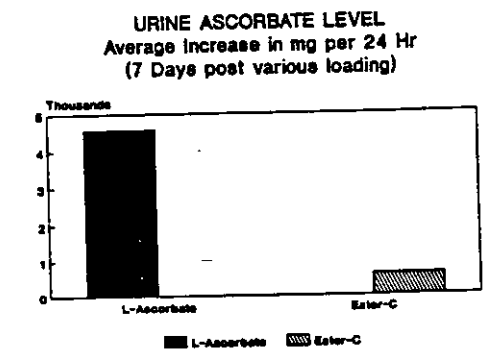



Figure 11.

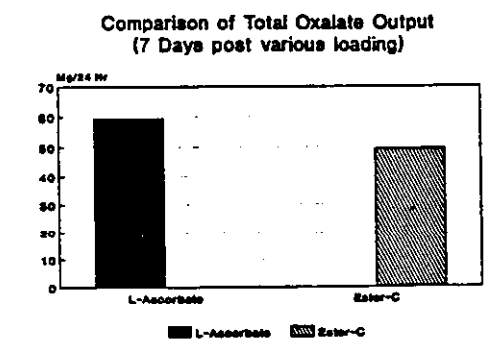



Figure 12.

Life Sciences, Vol. 48, pp. 2275-2281 Pergamon Press
Printed in the U.S.A.

# COMPARISON OF THE ANTI-SCORBUTIC ACTIVITY OF L-ASCORBIC ACID AND ESTER C® IN THE NON-ASCORBATE SYNTHESIZING OSTEOGENIC DISORDER SHIONOGI (ODS) RAT

Anthony J. Verlangieri, Michael J. Fay and Anthony W. Bannon

Atherosclerosis Research Laboratories
Department of Pharmacology
University of Mississippi, University, MS 38677

(Received in final form April 4, 1991)

## Summary

The Osteogenic Disorder Shionogi (ODS) rat, Clea Inc., Tokyo, Japan lacks the ability to synthesize L-ascorbic acid (AA). As with man, monkey and the guinea pig, this rat lacks L-gulonolactone oxidase necessary for the synthesis of AA from glucose. This study shows this animal to be an alternative to the guinea pig in AA studies. The anti-scorbutic potency of Ester C®(EC), a calcium ascorbate and calcium threonate mixture, was compared with an AA dose of equal ascorbate activity equivalents (AAE) for anti-scorbutic activity in the ODS rat. The minimal anti-scorbutic dose of EC was determined to be 0.44 mg/kg/day (AAE), while an AA dose of 0.51 mg/kg/day (AAE) was not anti-scorbutic in a 24 day study. At 24 days EC rats gained 125% of initial body weight (BW) and the AA rats only 45% BW. Scorbutic signs at 24 days were scored on a 0(min) to 3(max) scale. The EC/AA ratio scores were: hemorrhage 0/1.4, behavior change 0/2.0, piloerection 0/2.2, mobility 0.4/2.2, dysbasia 0.6/2.8 and ataxia 0.4/1.0. Pearson's correlation coefficient for BW versus AAE was r=.34 for the AA group and r = .90 for the EC group. The morbidity index for EC was 0/5 and for the AA group 2/5. The AAE dose of AA which was 16% higher/day than the EC AAE dose was not anti-scorbutic, while the EC dose was anti-scorbutic. EC rats had 3.5X greater weight gain, a sensitive indicator of scurvy, than the AA rats. EC rats had 3-4 times less, if any, scorbutic signs than AA rats. The results clearly show that, based on ascorbate activity equivalents, EC has more available ascorbate activity/potency than AA. The mechanism of this increased potency is believed to be due to the facilitated transport of AAE into the cell by the threonate (a normal *in vivo* metabolite of AA) present in the EC product. In addition, previous studies have shown EC (AAE) to be higher in plasma and excreted less rapidly than the AAE derived from AA administered orally.

Ester C®(EC) (U.S. patent no. 4822816, Inter-Cal Corporation, Prescott, AZ) is a complex mixture containing calcium ascorbate, dehydroascorbate (DHA), calcium threonate (CT), and trace levels of xylonate (CX), and lyxonate (CL). The ascorbate activity equivalent (AAE) of this product is 72% ascorbic acid activity, with 4% DHA, 1.3% CT and less than 0.1% of CX and CL each. The remainder of the product is composed of calcium (4.5%) and water of hydration. A normal metabolic product of L-ascorbic acid metabolism is CT (1). Bush and Verlangieri showed that EC administered at 10 mg/kg (AAE) versus ascorbic acid (AA) at AAE by gavage was absorbed more rapidly and excreted less rapidly in rats than AA (2). It was hypothesized that the mechanism of these observations may

0024-3205/91 $3.00 + .00
Copyright (c) 1991 Pergamon Press plc

have been due to the presence of CT or CX and possibly CL or their combination. More recently Fay and Verlangieri have shown that CT facilitates the uptake of $^{14}$C-ascorbic acid into T-cell lymphoma cells in culture. Maximum increase with CT was shown to be 177% greater than without CT (3). CT is produced in the manufacturing process of EC. Little is known about the biological activity of this compound other than when CT was administered to rats for 120 days there were no adverse effects on organ weights, biochemical parameters and survival times as compared to controls (4).

The Osteogenic Disorder Shionogi (ODS) rat is a mutant Wistar rat strain that has lost the ability to synthesize ascorbate (5) due to its lack of L-gulonolactone oxidase activity. Without dietary AA, these rats exhibit the signs of scurvy: weight loss or no gain, hemorrhage, behavior change, weakness, ataxia and limb weakness (dysbasia) (5). Other biochemical and physiological aberrations associated with AA deficiency and scurvy have also been noted in these rats (6,7,8).

The purpose of the present study was to compare the minimal anti-scorbutic doses (MAD) of EC and AA using equivalent ascorbate activity (AAE) of the two compounds in the ODS rat. Scurvy was assessed using the same criteria detailed above as well as those used with the guinea pig (9). The study was based on the theory derived from previous EC studies that the MAD of EC may be less than AA due to the facilitation or utilization of ascorbate activity produced by the metabolites in the EC product.

## Methods

Ten ODS rats were obtained from Clea, Inc., Tokyo, Japan. Upon arrival the body weights were recorded and the rats marked for identification. They were housed individually in plastic cages lined with bedding material and acclimated for 9 days. The animal facility was maintained at 22 $\pm$ 0.5°C with a 12:12h light:dark cycle. During the acclimation period rats were provided deionized water and Purina Rat Chow® ad lib. This diet contains only trace amounts of AA. Preliminary studies in our laboratory, as well as Clea, showed rats maintained on this type of diet become scorbutic in 2 to 3 weeks if not supplemented with AA. This 9 day period thus served to deplete AA stores prior to beginning the study.

At the end of this 9 day period the rats were randomly divided into 2 groups of 5 rats each. One group was supplemented with 0.06 mg AA/ml distilled drinking water and the other group supplemented with 0.08 mg Ester-C®(EC)/ml distilled drinking water. Based on analysis, 0.08 mg EC has ascorbate activity equivalents equal to 0.06 mg AA. Therefore, each drinking solution had the same AAE/ml. These concentrations were chosen from unpublished preliminary data.

Three days per 7 day week, fresh solutions of AA and EC were prepared and fluid intakes were recorded. At these times, body weights were also recorded and the rats were rated on a scorbutic rating scale (Fig. 1). Each score from the individual components (e.g., behavior, ataxia) was added together to obtain a total score. Absence of a sign was scored as zero except for bw gain. The minimum for this total score was 1 (least scorbutic) and the maximum was 19 (most scorbutic). The assessor remained the same for the duration of the study.

For statistical analysis, data taken from days 1, 5, 10, 14, 19 and 24 were used. Body weight and fluid intake data were analyzed using ANOVA followed by the student Newman-Keuls where appropriate. Data from the scorbutic rating scale were analyzed using the Mann-Whitney U procedure. T-tests were utilized for comparisons of mean intake of EC and AA expressed as AAE. Pearson's Correlation

was used to establish body weight gain relationships between the groups. The level of significance was set at $p<0.05$.

| | | Score |
|---|---|---|
| 1. | Hemorrhage | |
| | Initial red tinge around eyes/nose | 1 |
| | Defined red area | 2 |
| | Defined red area with cellular debris | 3 |
| 2. | Behavior | |
| | Exploratory behavior in open field | |
| | Slight decrease | 1 |
| | Moderate decrease | 2 |
| | Absence | 3 |
| 3. | Piloerection back/neck | |
| | Slight erection | 1 |
| | Moderate erection | 2 |
| | Significant erection | 3 |
| 4. | Weakness | |
| | Movement is slow | 1 |
| | Movement is slow/labored | 2 |
| | Ataxic | 3 |
| 5. | Ataxia | |
| | Absent | 0 |
| | Present | 1 |
| 6. | Dysbasia (limb weakness) | |
| | Slight | 1 |
| | Moderate | 2 |
| | Severe | 3 |
| 7. | Weight Change (Greater than 3 gms/2 days) | |
| | Gain | 1 |
| | Stable (no gain or loss) | 2 |
| | Loss | 3 |

FIG. I
Scorbutic Rating Scale

## Results

The growth curves (body weight) over the experimental period are shown in Fig. 2.

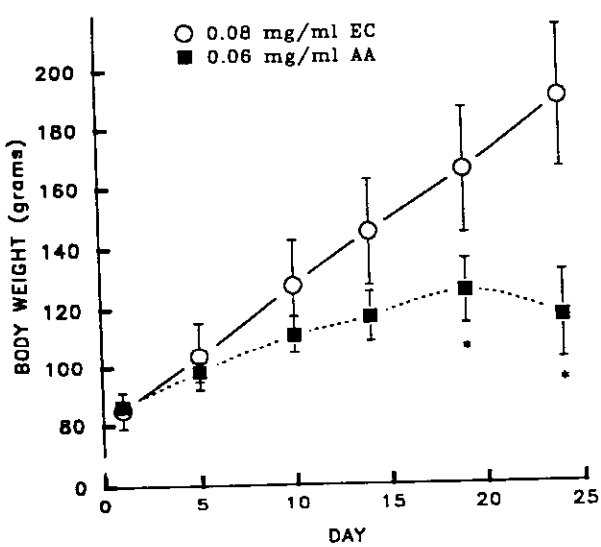



FIG. 2
Growth Curves for the AA and EC Supplemented Rats with S.E. bars

The EC group had a significantly greater increase in body weight (125%) compared to the AA group which only had a 46% increase in body weight. Water intakes of each group did not differ significantly (28 mls/day) and if intake is based on body weight, the AA group consumed 16% more AAE then the EC group (10.7 mg/kg EC vs. 12.4 mg/kg AA, total over the 24 day study).

The mean scorbutic rating for each parameter scored on days 5, 14, and 24 are shown below in Table I. Day 0 was rated 0 except for body weight.

Table I
Mean Individual Scorbutic Parameter Rating[1]

| | DAY 5 | | DAY 14 | | DAY 24 | |
|---|---|---|---|---|---|---|
| Parameter | GROUP EC | AA | GROUP EC | AA | GROUP EC | AA |
| Hemmorrhage* | 0.4(2)[2] | 0.8(4) | 0.0(0) | 0.4(1) | 0.0(0) | 1.4(3) |
| Behavior* | 0.6(2) | 1.2(4) | 0.2(1) | 1.2(3) | 0.0(0) | 2.0(3) |
| Piloerection* | 0.2(1) | 0.4(2) | 0.2(1) | 1.2(4) | 0.0(0) | 2.2(4) |
| Mobility* | 0.8(2) | 1.2(4) | 0.4(2) | 2.0(5) | 0.4(2) | 2.2(4) |
| Dysbasia* | 0.8(2) | 1.2(4) | 0.4(2) | 2.8(4) | 0.6(2) | 2.8(4) |
| Ataxia** | 0.4(2) | 0.8(4) | 0.4(2) | 1.0(5) | 0.4(2) | 1.0(5) |
| Weight Change*** | 1.4(2) | 1.2(1) | 1.0(0) | 2.0(3) | 1.0(0) | 2.2(3) |
| Mean Scores | 0.7 | 1.0 | 0.4 | 1.4 | 0.3 | 1.9 |

*Min. score = 1/max. score = 3; **Absent = 0/present = 1
***Weight gain = 1/no change = 2/weight loss = 3
[1]Sum of individual scores/N; [2]frequency of scores over minimum

Mean total scorbutic ratings over the entire course of the study are shown in Fig. 3

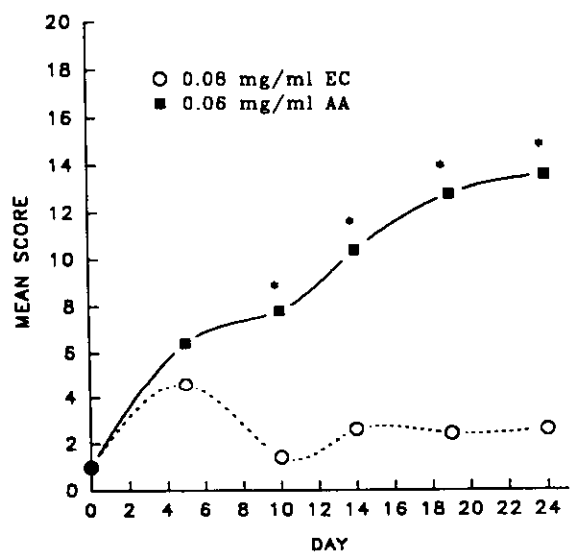



FIG. 3
Mean Total Scorbutic Ratings Over Entire Study (sum of all scores/N)

The AA group had consistently higher scores (more scorbutic) than the EC group. Statistical differences were found on days 10, 14, 19 and 24.

As shown in Fig. 4, Pearson's Correlation revealed no relationship between BW gain and AAE (mean total daily group intake in mgs) in the AA group. However, there was a significant high correlation between AAE (mgs) and body weight gain when the ascorbate equivalents were administered as Ester C® (Fig. 5).

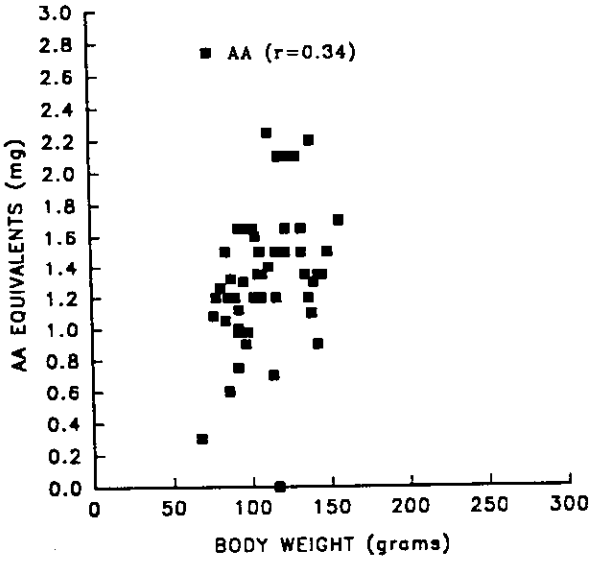



FIG. 4
Scatter Plot: AAE vs. BW for AA Group

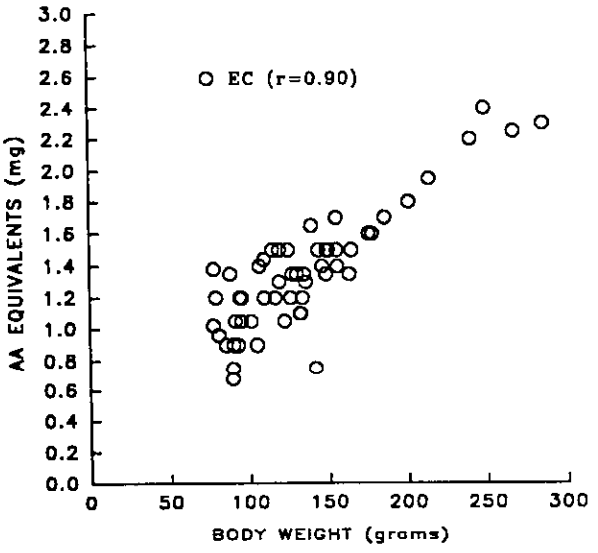



FIG. 5
Scatter Plot: AAE vs BW for EC Group

## Discussion

It is clearly shown in Fig. 2 that EC rats had normal continuous growth over the 24 day study, while the AA rats had a slower growth rate and began losing weight on day 19 of the study. On day 24 the EC rats had increased their BW by 125% and the AA rats only 45%. There were no significant differences in water intake (mls) per day between the groups over the 24 day period. The concentrations of EC and AA per ml of drinking water contained equal ascorbate activity equivalents (AAE) based on the EC Certificate of Analysis of ascorbate activity. The 4% DHA in the EC product was also taken into account since it may be reduced to AA in dependent species (10). The oxidation of EC and AA was not different at 48 hours by HPLC. Therefore, the actual concentrations as AAE were 0.06 mg/ml AA and 0.08 mg/ml EC in the drinking water. Based on BW over the duration of the study, the AA rats ingested 0.51 mgs AAE/kg/day and the EC rats 0.44 mg AAE/kg/day. The EC rats ingested 16% less ascorbate activity per day. The difference in growth rate is most dramatic, with EC increasing BW 3.5X, a clear anti-scorbutic effect. On the other hand, the AA dose used was not anti-scorbutic, demonstrated by less overall body weight gain, with a notable decrease on day 19.

The anti-scorbutic effect of EC is also evident from Fig. 3, showing that the AA group had a mean total scorbutic rating score over the 24 day period of 13, while the EC group score was only 3. The initial rise to 4 on day 5 in the EC group is evidence that the rats were becoming AA depleted on the Purina Chow®, and that EC repleted AA stores 4x more effectively than AA by day 24. Table I exemplifies the anti-scorbutic status of the EC rats. On the final day of the study, the AA rats had 5x more severe scorbutic signs than the EC rats. In addition, there were 2 animals in the AA group who were so morbid at day 24 that death was expected from scurvy within a day or two. There was no morbidity in the EC group. According to Pearson's correlation, there was no significant relationship between BW increase and AA intake in the AA group, but a strong ($r=0.90$) positive relationship between BW and EC intake in the EC group (see Figs. 4 and 5).

It is clear from this study that, based on ascorbate activity equivalents, EC is 4-5x more potent or effective than AA in preventing scurvy. The authors believe, based on previous reports and from the present results, that this increase is due to CT, and possibly CX and CL. Because CT is present in the highest concentration in the EC product, and because preliminary evidence suggests that it increases AA uptake in T-lymphoma cells (3), we believe threonate facilitates cellular uptake of AA, thereby increasing its potency compared to an equivalent ascorbate dose derived from AA.

The mechanism of this facilitation is unknown at the present time but is the subject of current investigation. Since the transport of AA has been shown to depend, in part, on insulin (11), and may involve an energy-dependent process, threonate may act in concert with insulin at the cellular receptor. This interaction may involve the glucose receptor specifically since it has been shown that glucose decreases AA uptake in heart endothelial cells (11) and that ascorbic acid transport is inhibited by the glucose transport inhibitor cytochalsin B (12).

Regardless of the mechanism of the increased potency of ascorbate activity when delivered as Ester-C® as compared to ascorbic acid the increase is significant. This study confirms that the presence of ascorbate metabolites enhance or facilitates the utilization of ascorbate activity in this scorbutic model.

## Acknowledgements

Supported by Inter-Cal Corporation, Prescott, AZ and overhead funds of the principal investigator, Dr. Anthony J. Verlangieri.

## References

1. M. LEVINE and M. KYOJI, Vitam. Horm. 42 1-64 (1985).
2. M.J. BUSH and A.J. VERLANGIERI, Res. Commun. Chem. Path. Pharmacol. 57 137-140 (1987).
3. MICHAEL J. FAY and ANTHONY J. VERLANGIERI, Pharmacol. Res., Submitted, (1991).
4. M. THOMAS and R.E. HUGHES, Fd. Chem. 17 79-83 (1985).
5. F. HORIO, K. OZAKI, A. YOSHIDA, S. MAKINO and Y. HAYASHI, J. Nutri. 115 1630-1640 (1985).
6. F. HORIO, K. OZAKI, M. KOHMURA, A. YOSHIDA, S. MAKINO, and A. YOSHIDA J. Nutr. 116(11) 2278-2289 (1986).
7. F. HORIO, K. OZAKI, H. ODA, S. MAKINO, Y. HAVASHI and A. YOSHIDA, J. Nutr. 117(6) 1036-1044 (1987).
8. S. MORI, Y. TAKEUCHI, M. TOYAMA, S. MAKINO, T. HARAUCHI, Y. KURATA, and S. FUKUSHIMA, Cancer Letters 38(3) 275-282 (1988).
9. R.O. MUMMA, E.E. McKEE, A.J. VERLANGIERI and G.P. BARRON, Nutr. Reports, Intern. 6 133-137 (1972).
10. RICHARD C. ROSE, B.B.A. 947 335-366 (1988).
11. J.C. KAPEGHIAN and A.J. VERLANGIERI, Life Sci. 34 577-584 (1984).
12. M.J. FAY, M.J. BUSH and A.J. VERLANGIERI, Life Sci. 46 619-624 (1990).

# Encouraging Response in Horses with Degenerative Joint Disease Treated with Ester-C® Calcium Ascorbate

Degenerative joint disease is a crippling syndrome that may affect horses of any age and at all levels of athletic competition. The economic effects of degenerative joint disease in athletic horses can be considerable. The expensive investment in the horse itself and the years of careful training and conditioning can all be lost when a horse is affected by crippling joint problems. And that loss is amplified by the added cost of extensive veterinary treatment along with the emotional trauma of watching a well-loved companion suffer.

Degenerative joint disease in horses, such as ringbone, hock spavin, osteochondrosis dissecans, and other crippling conditions, is generally considered progressive and incurable. Most horses become steadily worse with time and continued work. Although some horses may remain useful for light riding, any serious athletic endeavor such as racing, jumping, reining, endurance, or dressage, is commonly ruled out. Veterinary therapy is often limited to pain suppression or joint surgery with the objective of destroying joint movement in order to alleviate pain. A large number of horses remain unsound despite traditionally accepted therapy.

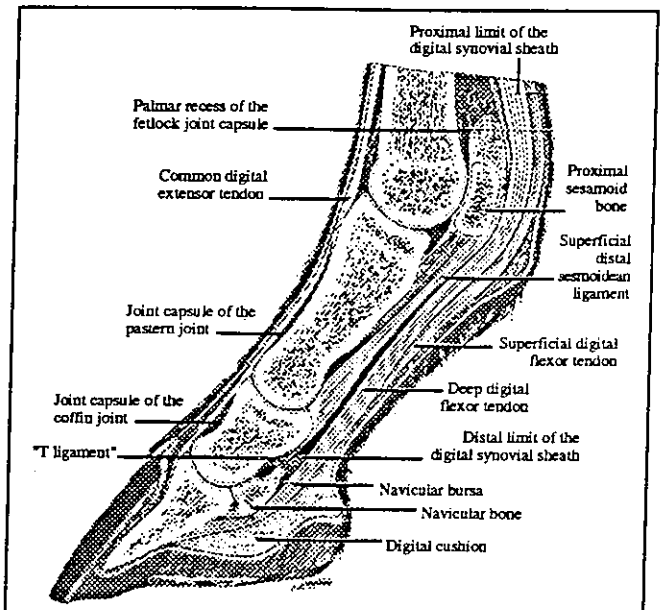

Proximal limit of the digital synovial sheath
Palmar recess of the fetlock joint capsule
Common digital extensor tendon
Proximal sesamoid bone
Superficial distal sesamoidean ligament
Joint capsule of the pastern joint
Superficial digital flexor tendon
Deep digital flexor tendon
Joint capsule of the coffin joint
"T ligament"
Distal limit of the digital synovial sheath
Navicular bursa
Navicular bone
Digital cushion

*Fig. 1. Sites of Ringbone (periarticular and degenerative osteoarthritis)*

Horse owners seeking relief for their animals and a return to useful performance often seek non-traditional remedies. Modalities such as acupuncture, cold laser, and magnetic field therapy have been tried, as well as use of nutritional supplements such as glycosaminoglygans (GAG) and chondroitin sulfates, hyaluronic acid, methyl sulfonylmethane (MSM), and dimethyl glycine (DMG). Racehorse trainers have advocated feeding pure ascorbic acid. With all of these treatments, the percentage of horses improved to soundness is about the same as with traditional methods; that is, a large number of horses, particularly the more severely affected, continue to deteriorate.

Vitamin C, or ascorbic acid, has been shown to play a role in a number of biochemical reactions in the metabolism of collagen, in the immune system, and as an anti-oxidant, binding free-radicals which are a factor in inflammation and pain. Although most animals other than primates are able to synthesize ascorbic acid in their bodies, the production of adequate levels may be affected by infection, trauma, or stress. For many years ascorbic acid has been advocated for the prevention or treatment of many conditions in humans, including those affecting joints, such as arthritis. Unfortunately, pure ascorbic acid is rapidly excreted from the body, often before it can be transported to the needy tissues and joints, and its acidity causes irritation to the gastrointestinal tract.

A patented form of Vitamin C has been developed by Inter-Cal Corporation of Prescott, Arizona, under the name Ester-C® calcium ascorbate. Ester-C® is a non-acidic product that is rapidly absorbed from the intestinal tract and excreted more slowly from the body than ascorbic acid. It also crosses cell membranes more efficiently than ascorbic acid which results in significantly higher intracellular levels. Metabolism of the calcium ascorbate produces ascorbic acid and several significant metabolites which also exhibit positive effects.

Observations in humans show that Ester-C® calcium ascorbate lessens symptoms of pain and stiffness. Similiar observations were made in dogs in studies by Charles Dockter, D.V.M., Prescott, Arizona and Geir Erik Berge, a veterinarian in Oslo, Norway. Both veterinarians reported that about 75% of the patients treated with Ester-C® showed clinical improvement.

N. Lee Newman, D.V.M., while in practice in Bullhead City, AZ., became aware of Dr. Dockter's work in dogs. She achieved similiar results in dogs in her practice, and saw the potential for Ester-C® in treatment of joint disorders in horses. The first horse

she tried the product on was a Quarter horse mare retired from the show ring to the broodmare band because of arthritis in her shoulders and knees. When Ester-C® was added to her feed, the mare became sound within a short period of time, continued sound, and was able to return to active riding and showing. Dr. Newman later used Ester-C® in several other horses either exhibiting signs of joint disorders, myositis, musculoskeletal trauma, or prophylactically in two-year-old racehorses. Most horses showed good to excellent responses to Ester-C®.

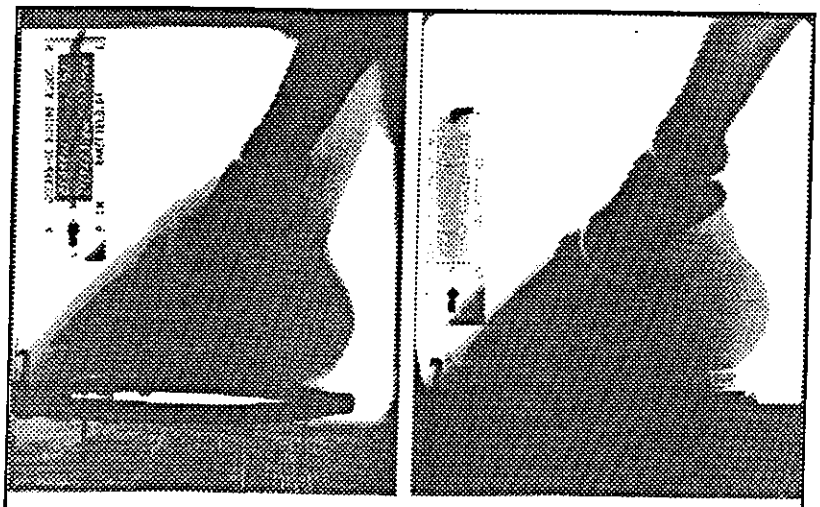


Fig. 2. June '91- Cherry Queen - periarticular ringbone at P2-P3 pastern joint.

Jan. '92 -Cherry Queeen reported to be 100% sound after Ester-C® therapy.

Formal clinical trials with Ester-C® in horses selected for joint disorders, particularly degenerative joint disease, were undertaken in 1992 by Dr. Newman. Horses were selected which had lameness due to skeletal or joint disorders which could be confirmed radiographically as well as by veterinary examination. Horses with particularly severe conditions, and those which had failed to improve in condition over a prolonged period, despite other therapies or rest, were chosen, as well as less severely affected horses. Several horses were already on various therapies for their problems, including phenylbutezone, Adequan, MSM, and corrective trimming/shoeing.

Over the course of the trials, ranging from two months to ten months in total length, all of the subjects were taken off any other therapy except Ester-C®. The dose of Ester-C® ranged from 9.9 grams to 30.00 grams of ascorbic acid equivalent (20-60 mg/kg). Of the ten horses observed, nine of them (90%) showed good to excellent response to Ester-C®. Only one horse failed to improve in degree of soundness and pain relief. Some horses, which had not been rideable unless heavily medicated with pain suppressing drugs, are currently sound enough to be ridden regularly. Of the ten horses, two are no longer on Ester-C® or any other medication, and are being used normally by their owners. Another two have made remarkable progress, considering the severity and duration of joint disease: one horse with marked degenerative bone spavin in both hocks is being ridden and another with a traumatic dislocation in the pastern joint and severe ringbone is once more pasture-sound. Both continue to improve with Ester-C®. The remaining horses in the study continue to be followed and indicate steady improvement. In addition to the clinical improvement in soundness, radiographic studies of some horses show an improved picture in joint structure, with reduction in inflammation, joint destruction, bone lysis, and periosteal exostosis.

No adverse reactions or side effects were seen in any horses. Subjects consumed the Ester-C® powder readily when mixed in their regular ration.

In conclusion, this study shows that administration of oral Ester-C® calcium ascorbate gives symptomatic relief of degenerative joint disorders in horses, allowing a return to functional status as riding horses in a high percentage of horses studied. Several horses had been unsound for prolonged periods (one to two years) or unresponsive to other forms of therapy, yet showed rapid relief from pain with Ester-C®, some within just a few weeks. Thus, Ester-C® should prove to be a good alternative for treatment of degenerative joint disorders in horses, without apparent side effects or toxicities. Further investigations are in progress, and subjects will be monitored for several months to further clarify the responses. Contact N. Lee Newman, D.V.M., for further information on her clinical trials, P.O. Box 255, Middletown, VA 22645.

**Selected References**


1. Adams, O.R.: Lameness in Horses. 3rd Ed.Philadelphia, Lea & Febiger, 1974, p. 359.
2. Colahan, P.T., Wheat, J.D., and Meagher, D.M.: Treatment of middle phalangeal fractures in the horse. J. Am. Vet. Med. Assoc., 178:1182, 1981
3. Facklemen, G.E., and Nunamker,D.M.: Manual of Internal Fixation. New York, Springer-Verlag 1982, p.74.
4. Fesseler, J.F., and Amstutz, H.E.: Fracture Repair. In Large Animal Surgery. Edited by F. W. Oehme, and J.E. Prier. Baltimore, Willaims & Wilkins Co.. 1974, p.260.
5. Haakenstad, L.H.: Investigations on Ringbone. Nord. Vet. Med., 7:1, 1954

ANIMAL NUTRITION
SPRING 1993 VOL.18

# Ester-C® Calcium Ascorbate Eases Lameness in Horses

A clinical trial by Dr. N. Lee Newman, D.V.M., has shown that oral administration of patented **Ester-C®** calcium ascorbate provides symptomatic relief of pain and lameness associated with degenerative joint disorders in horses. *Ninety percent (90%) of horses treated with Ester-C® for moderate to severe cases of ringbone, bone spavin, and other joint disorders showed good to excellent responses.*

Ascorbic acid, or Vitamin C, has been reported to alleviate symptoms of pain and stiffness in humans, and in dogs with arthritic and degenerative joint problems. Now, results of the first year of studies in horses show that an improved, patented form of Vitamin C may help return horses suffering from degenerative or traumatic joint disorders to riding soundness.

Pure ascorbic acid is rapidly removed from the body, often before it has time to reach joints and other tissues. A patented combination of ascorbate with certain minerals, **Ester-C®**, is absorbed in the body up to four times more efficiently than conventional Vitamin C, and its metabolism at the cellular level produces higher levels of ascorbate.

Horses with moderate to severe degenerative joint disorders were given **Ester-C®** powder mixed in their daily feed. *Within a short period of time, 9 out of 10 (90%) horses showed remarkable improvement in soundness and pain relief.* Horses which had been unrideable for periods of up to two years because of joint disease became sound enough to return to regular under-saddle use, and horses which were so severely affected as to warrant consideration of humane euthanasia were relieved of pain and returned to a more normal life.

Traditional therapy for degenerative joint disorders in horses has only a low success rate, with many horses becoming progressively worse despite treatment. Non-traditional treatments such as acupuncture, laser or magnetic therapy have no better success rate. **Ester-C®** has shown a 90% improvement rate in seriously lame horses, with no adverse reactions or side effects.

*Fig. 1. Charles Dockter, D.V.M. reviews Dr. Newman's radiographs of Cherry Queen.*

Cindy Hall credits **Ester-C®** with saving the life of her Morgan, Mischief. "Before **Ester-C®**, Mischief was in so much pain and misery, I planned to have him put down. He hated life, had turned vicious toward anyone approaching him to the extent he had to wear a muzzle in the stable, and could barely hobble around. Today, he is a different horse. He runs and plays in pasture, is affectionate and friendly, and is even being ridden again. This 'gold dust' has given me back my horse."

New Yorker was a promising show horse when he became lame from poor shoeing and developed an enlargement of his right front sidebone to the point it pressed on the joints. Two years of rest and corrective trimming failed to alleviate New Yorker's pain enough that he could be ridden without large doses of phenylbutezone ("Bute"). Within just a few weeks of being on **Ester-C®**, New Yorker was being ridden without his dose of "Bute", and after six months is now sound and able to be shown without any medication at all. He has a promising future as a junior hunter-dressage mount.

Stardust and Quizkid were both victims of traumatic falls, with severe injuries to joints. Leo's hocks gave in because of his poor conformation. Cherry Queen became severely lame from ringbone, Sheba suffered acute laminitis, and Ethel has cysts in her hock bones. All have improved in soundness and pain relief on **Ester-C®**. Even those horses on pain medications when **Ester-C®** was started have been able to discontinue their use, and continue to improve on **Ester-C®** alone.

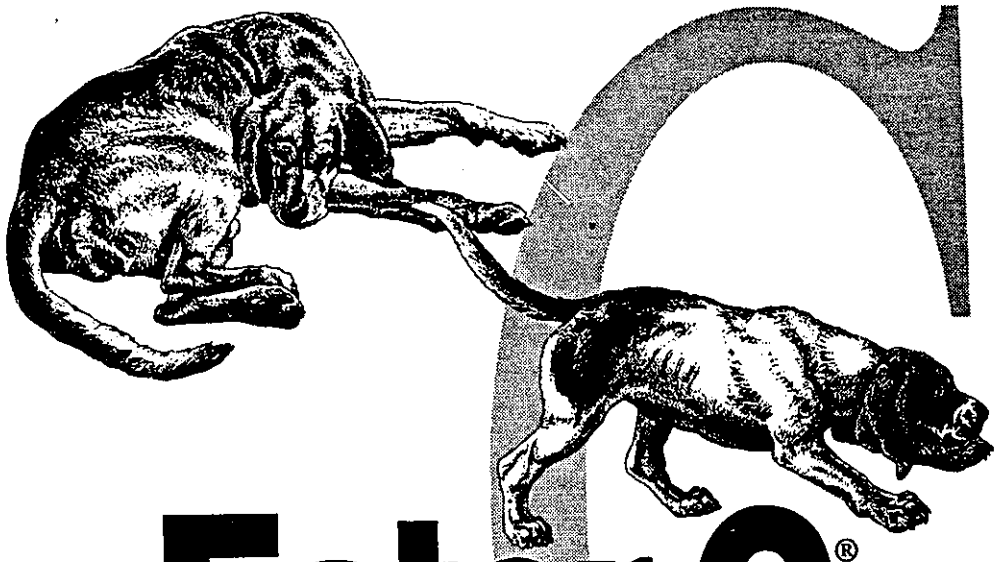
**Ester-C®** is a promising new treatment for joint lameness of horses. While research goes on to further identify its uses and effects, there are nine horses out there today who are running with less pain and stiffness because of this nuritional supplement.

Contact N. Lee Newman, D.V.M., for further information on her clinical trials, P.O.Box 255, Middletown, VA 22645.

Turn The Page For...

*Feline Festivities...*

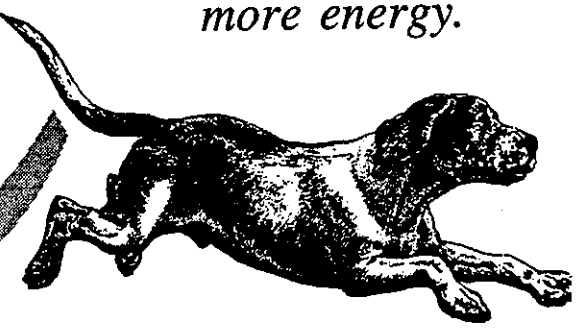
*What's Really In Your Pet's Food?*



*"Tahoe ... marked change in behavior... ...climbed stairs with comparative ease... eager to run... more playful... more energy."*

# Ester-C®

# For Joint Discomfort - A Study



**by L. Phillips Brown, DVM**

Cartilage consists of collagen, proteoglycans and water. Collagen is a tough, stringy protein that physically connects tissues and provides tensile strength to cartilage. Proteoglycans trap and hold water, allowing for compression resistance. Vitamin C (ascorbic acid) is critical for maintaining the physical integrity of collagen. In normal cartilage, the anabolic and catabolic processes of chondrocyte cells are in equilibrium. In degenerative (i.e. aging) or inflammatory conditions,the breakdown processes are excessive,often causing stiffness and soreness.

Because humans (and other species, such as guinea pigs) cannot manufacture endogenous ascorbic acid, vitamin C must be present in their daily diet. In fact, millions of people routinely supplement their diets with vitamin C in one form or another.

Dogs, on the other hand, can manufacture ascorbic acid and theoretically should not require supplemental vitamin C. However, earlier studies in dogs by Belfield (1976) and Kronfeld (1989) and later by Berge (1990) and Docktor (1989), demonstrated that supplemental vitamin C might be beneficial in reducing chronic inflammation. Unfortunately, many animals cannot tolerate the high levels of ordinary vitamin C needed to provide beneficial results.

Dogs, horses and even cats may not manufacture sufficient quantities of ascorbic acid to counter the effects of aging, stress, breed predilections to inherited dysfunctions, environmental irritants and pollutants and poor quality or high fat pet foods. A form of vitamin C that would provide higher intracellular levels without causing any gastrointestinal side effects would be a useful and unique product.

**Product Background**

According to the manufacturer, Inter-Cal Corporation, of Prescott, Arizona, Ester-C® is a patented ascorbate supplement that contains calcium ascorbate, naturally occurring dehydroascorbate and the vitamin C metabolite, threonate. Threonate permits ascorbate to be more rapidly absorbed from the gastrointestinal tract, cross cell membranes more efficiently, reach higher cellular levels and be excreted more slowly than ordinary vitamin C. Ester-C® ascorbate is pH neutral and believed to be water and, to some degree, fat soluble.

**Mechanism of Action**

Vitamin C may act as an immuno-responsive and chondro-regenerative agent. In degenerative or inflammatory conditions, excessive collagen breakdown results in joint discomfort and skeletal changes. Ester-C® calcium ascorbate provides high and prolonged levels of intracellular ascorbate and helps increase compression resistance of cartilage, mobilizes white blood cells to the site of inflammation and enhances prostaglandin synthesis. The net result is increased comfort and mobility.

(continued onpage 2

**Clinical Studies**

The study was conducted at the Best Friends Animal Santuary in Kanab, Utah.

Different ascorbic acid (vitamin C) preparations, identified only by code, were fed twice daily to each group of dogs (phase I) and then discontinued for 3 weeks.

During the next 4 weeks (phase II), each group was fed a preparation different from the one received during phase I, followed by a 3 week discontinuance.

During phase III, some groups received the same preparation they previously received during phase I, while others received a preparation not previously fed.

---

"Mitzie...
more active...
able to walk up steps..
walks up on her toes again ...
no other prescription drugs
before or during."

---

**Results**

The response of each dog in each group was measured by changes in comfort and mobility. Responses were graded on a scale of zero (no response) to three (very good).

*The Average Mobility Improvement Score* (Table 1) was calculated by adding the total number value of each response (0-3) divided by the total number of animals responding to a particular preparation.

(continued onpage 27)

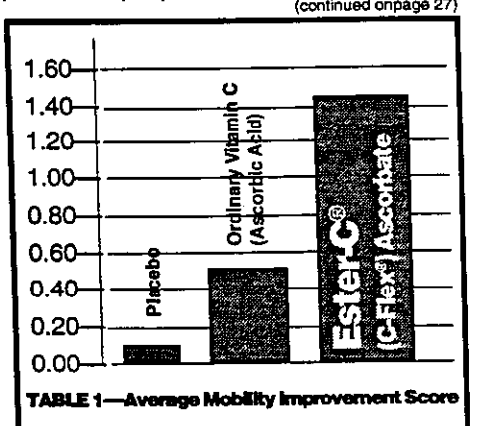
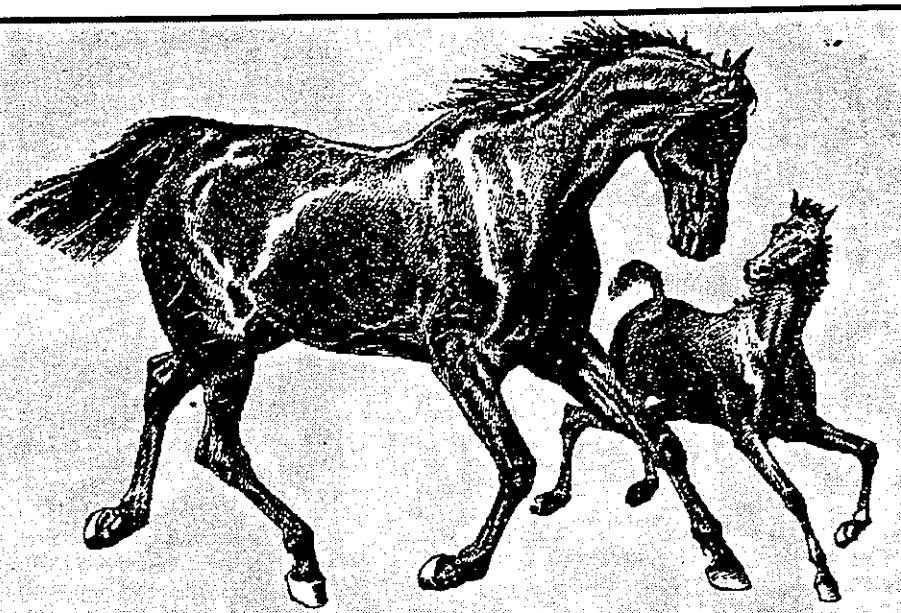



TABLE 1—Average Mobility Improvement Score



# VITAMIN C FOR HORSES

In an equine study conducted over two years by veterinarian N. Lee Newman, dramatic improvements in pain relief and soundness were observed in 90% of horses with severe, progressive joint or bone disorders which were administered Ester-C® calcium ascorbate. All the horses in this trial were diagnosed with chronic and progressive degenerative arthritis, traumatic joint injuries, developmental bone disease, or laminitis. Most were in end-stage conditions. Traditional therapies were unsuccessful in alleviating lameness, damage was severe and progressive, and had been on-going for periods of up to several years.

"...dramatic improvements,
in pain relief
and soundness,
were observed
in 90% of horses
with severe,
progressive joint
or bone disorders
which were administered
Ester-C® calcium ascorbate"

Owners of the horses in this study were unable to ride and enjoy their horses in a normal manner, and had to watch them suffer pain and disability. Temperament changes—irritability, reluctance to be handled, even outright aggressive behavior — were commonly associated with these painful disorders.

When Ester-C® calcium ascorbate was administered, noticeable improvement in soundness, pain relief, and attitude was evident within a few days to weeks, and within 30 days of starting Ester-C®, all other medications, including pain and anti-inflammatory drugs, such as phenylbutezone and Adequan, had been stopped. The horses continued to improve over time, with 90% achieving good to excellent improvement within six months. Several horses which had been completely unsound recovered and are being ridden on a regular basis.

Two horses with such severe lameness, unresponsive to other therapy, that they were slated for euthanasia, have been able to return to comfortable lives and even light riding.

For additional information, Dr. Newman may be contacted at P.O.Box 255, Middletown, VA 22645.

## Conclusion

The findings suggest that although dogs can manufacture endogenous ascorbic acid, the amount produced may not be sufficient to prevent or counteract stresses associated with injury, aging or joint malpositioning. Low or declining ascorbic acid availability within the chondrocytes may compromise collagen homeostasis, resulting in a loss of tissue elasticity and compression resistance. Clinical signs of stiffness, discomfort or decreased mobility may be reversed by high levels of intracellular vitamin C achieved with Ester-C® calcium ascorbate.

The consistency and degree of response to Ester-C® calcium ascorbate suggests that it may be more beneficial in treating nonspecific locomotor discomfort than ordinary vitamin C, without signs of gastrointestinal irritation. The deterioration of "improved dogs" upon discontinuance of Ester-C® further verified it's usefulness as primary or adjunctive therapy.

From the results of this study, there is sufficient evidence to recommend oral Ester-C® calcium ascorbate to alleviate the pain and discomfort associated with certain musculoskeletal dysfunctions of dogs.

---

"Lucky...
marked difference...
doesn't hesitate
to join in on
the activities...
doesn't limp at all...
my "Happy-go-lucky" guy
once again."

## Biography

Dr. Brown is a graduate of the School of Veterinary Medicine of the University of California at Davis. A small animal clinician for over twenty years, Dr. Brown currently consults for several companies that manufacture and market natural products for companion animals. For further information on the clinical studies, contact Dr. Brown at P.O. Box 1177, St. Augustine, FL 32085.

---

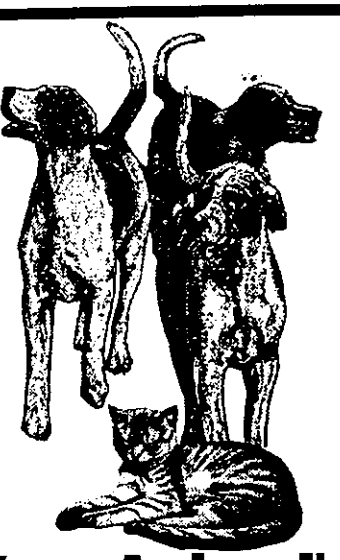
"Tippy...
gradually
began using the leg
and eventually
had full use of it."

---

## References


- Adkins, T.O. and Kronfeld, D.S.:Diet of racing and sled dogs affects erythrocyte depression by stress. Canadian Vet. J., 23:260-3, 1982.
- Allen, T.A. and Hand, M.S.: Conditionally Essential Nutrients. Proc.8th ACVIM Forum, pp.809-811, 1990.
- Belfield, W.O.: Chronic Subclinical Scurvy and Canine Hip Dysplasia. Veterinary Medicine/Small Animal Clinician. 1399-1401, 1976.
- Berge, G.E.: Polyascorbate (C-Flex), an interesting alternative by problems in support and movement apparatus in dogs. Norwegian Veterinary Journal. 102:579-581, 1990.
- Docktor, C.: unpublished data.
- Donoghue, S., Kronfeld, D.S., and Banta, C.A.: A possible vitamin C requirements in racing sled dogs fed a high fat diet. In: Nutrition,Malnutrition and Diet in Dogs and Cats ed. by Meyer, H. and Kienzie, E. pp.110-114, Hanover: Tierarziliche Hochschule, 1988.
- Fay, M.J.: Possible effects of ascorbic acid metabolites on the cellular uptake of ascorbic acid and other compounds. A dissertation for degree of Doctor of Philosophy, University of Mississippi, 1992.
- Goodman, S., Vitamin C: The Master Nutrient, Keats Publishing Inc., Connecticut, 1991.
- Kronfeld, D.S.: Stress supplements: Protein and vitamin C. Purebred Dogs/Kennel Gazette 100. 10:8-9, 1983
- Lewis, D.L., Morris, Jr. M.L., and Hand, M.S.,: Small Animal Clinician Nutrition Mark Morris Assoc., Kansas, 1990.
- Newman, N.L., unpublished data.
- Smith, C.: More on Vitamin C. Natural Pet. Vol 2, No.6:18-19, 1993.
- Stein, D. Natural Healing for Dogs and cats, The Crossing Press, California, 1993.





**Your Animal's Best Friend!**

**Ester-C®**

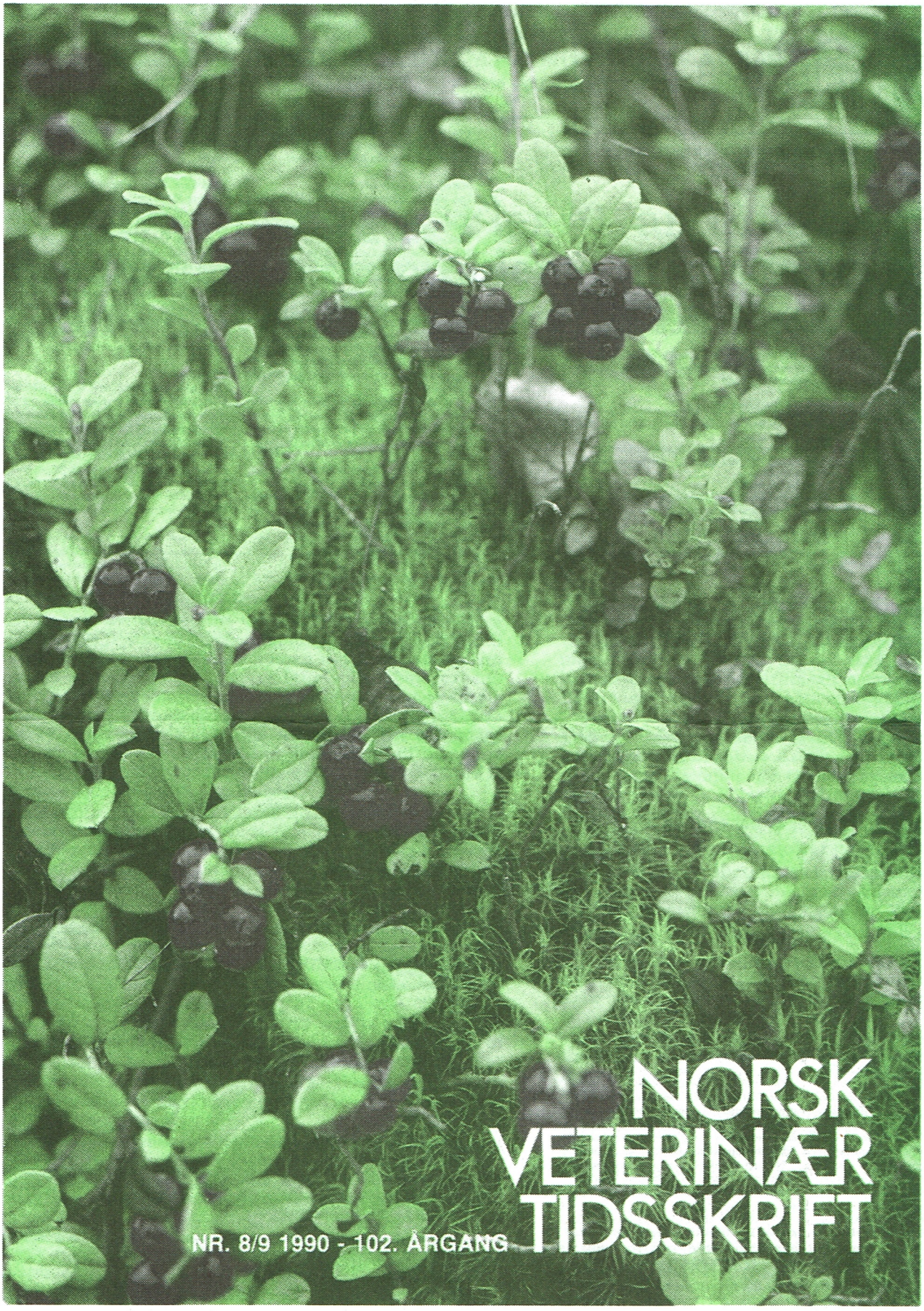
**C-Flex® Ascorbate**

- *Gentle (pH neutral)*
- *Maximum Absorption*

**Results!**

**Dynamite Marketing**
208-939-1557
**Natural Animal**
800-274-7387
**Natural Life Pet Products**
800-367-2391
**Precise Pet Products**
800-446-7148

Ester-C® and C-Flex® are licensed trademarks of Inter-Cal Corporation, Prescott, AZ 86301




**The Norwegian Veterinary Journal**, Volume 102, August/September 1990

**POLYASCORBATE (C-FLEX®), AN INTERESTING ALTERNATIVE BY PROBLEMS IN THE SUPPORT AND MOVEMENT APPARATUS IN DOGS**

*Clinical trial of ESTER-C® POLYASCORBATE in canines*

ISSN 0332-5741

NORSK VETERINÆR TIDSSKRIFT

DEN NORSKE VETERINÆRFORENING
SEKRETARIATET:
Sognsv. 4, 0451 Oslo 4
Tlf. (02) 46 05 14
Telefax (02) 69 04 50
Kontortid:
15.9.–15.5. 0800–1546
15.5.–15.9. 0800–1500
PRESIDENT:
Byveterinær
Herlev G. Bjørnøy
Måltrostveien 2
6400 Molde
Tlf. (072) 12 640
GENERALSEKRETÆR:
Veterinær Svein Kvaløy
Tokerudberget 19
0986 Oslo 9
Tlf. (02) 10 20 64
ASS. GENERALSEKRETÆR:
Jan Terjer Hanssen
Von Øtkensvei 32
1430 Ås
Tlf. (09) 94 11 06
NORSK VETERINÆRTIDSSKRIFT:
Redaktør:
Informasjonssjef Ulf Erik Gustavsen
Redaksjonssekretær:
Bente Sofie Nessan
FAGLIGE MEDARBEIDERE:
Professor Magne Yndestad
Professor Øystein Sjaastad
Avdelingsveterinær Steiner Waage
Professor Jorunn Grendalen
ANNONSER:
Den Norske Veterinærforening
Sognsveien 4
0451 Oslo 4
Tlf. (02) 46 05 13 (02) 46 05 14
Telefax (02) 69 04 50
TRYKKERI:
Hellstrøm & Nordahls
Boktrykkeri As
Grønlandsleiret 37
0190 Oslo 1
Tlf. (02) 17 39 30
Telefax (02) 17 20 63

ORGAN FOR DEN NORSKE VETERINÆRFORENING
NR. 8/9 – 1990 – 102. ÅRGANG

LEDER

571 Minsaas A. Etikk - mål eller middel?

FAGARTIKLER

575 Bjerkås E. Persisterende hyperplastisk tunica vasculosa lentis/persisterende hyperplastisk primær vitreus (PHTVL/PHPV). En arvelig øyesykdom hos dobermann.
579 Berge GE. Polyascorbat, (C-Flex®), et behandlingsalternativ ved kroniske forandringer i støtte- og bevegelsesapparatet hos hund.

FOR OG FRA PRAKSIS

583 Arnemo JM. Bruk av xylazin - Rompun® til epiduralanalgesi på storfe.
584 Waage S. Viral haermorrhagic disease hos kaniner.
585 Felleskatalog for veterinærer - spørreundersøkelse blant veterinærer.
587 CEM positive hester.
587 Klipp fra kort om forskning og landbruk nr. 4/90.
587 Waage S. Bekjempelse av storfe-leukose i Sverige.

FORUM FOR SMÅDYRPRAKTIKERE

589 Prestasjonsdjur/sällskapsdjur i relation till etik och djurskydd.

MILJØ OG HYGIENE

591 Engebretsen O, Helland B. Matforgiftning - Staphylococcus aureus enterotoksiner.
592 Klipp fra kort om forskning og landbruk nr. 5/90.
593 Kaupang K, Skjerve E. Djevler og kannibaler i Belgia.



# Polyascorbat, (C-Flex®), et behandlingsalternativ ved kroniske forandringer i støtte og bevegelsesapparatet hos hund.

Geir Erik Berge
Groruddalen dyreklinikk
Oslo Norge

**Innledning**
Helt siden Dr. Lind i 1740 oppdaget at saft fra sitrusfrukter kunne forebygge skjørbuk, har vitamin-C hatt en selvfølgelig plass i human ernæring. Alle dyrearter bortsett fra primater, marsvin, visse flaggermus-arter, laksefisker, insekt- og bløtdyr-arter, har enzymsystemer som omdanner glukose til askorbinsyre (1). Vitamin-C har derfor fått liten oppmerksomhet innen veterinærmedisin. Imidlertid er antagelsen om at dyr til enhver tid produserer tilstrekkelige mengder, neppe holdbar.

Askorbinsyre inngår i et stort antall biokjemiske reaksjoner innen kollagenstoffskiftet og immunapparatet, samt en serie med red-oks-reaksjoner.

Også metabolitter av vitamin-C er biologisk aktive.
I dagens forskning vies disse stor oppmerksomhet. Dannelsen antas blant annet å være avhengig av den intracellulære askorbinsyrekonsentrasjon.Under visse tilstander, som infektiøs- traumatisk-eller fysisk- stress, forbrukes større mengder askorbinsyre og dets metabolitter i de forskjellige vev. Under slike forhold kan det tenkes at kroppens egenproduksjon ikke er stor nok til å tilføre alle vev optimale mengder.

C-vitamin er et vannløslig molekyl som etter inntak skilles svært raskt ut gjennom nyrene. Askorbinsyre har pka = 4,17.

Det ideelle «C-vitamin-molekylet» vil være et syrenøytralt molekyl som ikke irriterer gastrointestinaltraktus, som absorberes raskt fra tarmen, og utskilles langsommere. Videre må det transporteres hurtig over cellemembraner slik at høy intracellulær konsentrasjon oppnås.

Nettopp et slikt C-vitamin er utviklet og patentert av Inter-Cal Corporation, Prescott, Arizona, under betegnelsen *poly-askorbat*, og registrert som C-FLEX®

Poly-askorbat er en kompleks blanding av kalsium askorbatmolekyler og de før omtalte metabolitter. I vandig løsning har polyaskorbat nøytral pH og det påvirker osmolariteten i tarmen mindre enn vanlig askorbinsyre.
Stoffet absorberes raskere fra fordøyelseskanalen enn vanlig C-vitamin hos både dyr og mennesker.
Videre oppnås en langsommere ekskresjon og høyere intracellulære konsentrasjoner. Kliniske undersøkelser indikerer at metabolittene har betydning for det økte intracellulære opptak av askorbinsyre. (2,3,4)

Davis observerte at poly-askorbat minsket både smerte og stivhet hos leddgiktpasienter



(Rapport til Inter-Cal 1988/Personlig meddelelse 1989). Indikasjoner for tilsvarende effekter ved inflammatoriske prosesser hos hund ble påvist av Dokter (Rapport til Inter-Cal 1988/ Personlig meddelelse 1989). Det antas at stoffskiftet i bindevev og bruskvev stimuleres og optimaliseres.

Hensikten med denne undersøkelsen var å vurdere polyaskorbats effekt på de kliniske symptomer ved kroniske lidelser i ledd, skjelett og muskulatur hos hund, som vanligvis behandles med antiflogistika eller corticoider.

**Materiale og metoder:**
Den kliniske utprøving er foretatt blant annet ved Groruddalen dyreklinikk over en seksmåneders periode i 1988/89. Hundre hunder av forskjellig rase og alder ble gitt ca. 30 mg/kg polyaskorbat (C-flex) oralt 3 ganger daglig.

Samtlige behandlinger ble gitt som følge av at eiere oppfattet dyrets symptomer som et problem i form av halthet, nedsatt bevegelighet og/ eller smerte. Diagnose ble stillet ved sykehistorie, klinisk undersøkelse og om nødvendig røntgen. Effekt ble vurdert dels ut fra klinisk undersøkelse, dels ved eiers vurdering av problemet. En vurderte behandlingens effekt etter 7-10 dager, etter 6 uker og hos de tilgjengelige etter ca 6 måneder.

Ved akutte lidelser og tilstander som raskt endrer symptombilde, er det vanskelig å skille mellom effekt av preparatet og andre faktorer. Slike pasientene ble derfor utelukket fra undersøkelsen.
En begrenser seg i denne artikkelen til å se på effekten på lidelser med
-: Symptomer som har en kjent årsak som forblir permanent, og
-: hvor symptomene også hadde vært stabile over et lengre tidsrom (minimum 6 uker) og
-: må forventes å vedvare.

Dyr med følgende kroniske lidelser ble tatt med:

Artroser
Spondyloser
Hofteleddsdysplasi
Eldre diskusprolaps med permanente sekundærforandringer
Senile slitasjeforandringer i støtte- og bevegelses-apparat

**Resultater:**
Effekten av behandlingen på hele hundematerialet er vist i tabell 1.
Behandlingsresultat relatert til de ulike diagnosene er vist i tabell 2.
Det var ingen klare forskjeller mellom de ulike lidelsene med hensyn til behandlingsresultat.

Det ble ikke påvist bivirkninger av behandlingen.

C-flex ble tilført både som pulverform og tablettform. Dette syntes ikke å ha noen innvirkning på effekten av stoffet.

I samråd med en del hunde-eiere ble seks hunder med kroniske artroseforandringer delt i to grupper. Gruppe en fikk C-flex, gruppe to fikk placebo. Etter en uke viste alle hundene i gruppe en bedring, mens ingen i gruppe to viste noen endringer i symptombildet. Behandlingen ble seponert i fire uker. Gruppe en fikk deretter placebo, mens gruppe to fikk C-flex. Etter en uke viste alle hundene i gruppe to bedring, og ingen i gruppe en. Det ble deretter foretatt ny «cross over» med samme resultat.

**Konklusjon:**
Dette tallmaterialet indikerer at poly-askorbat tilført oralt til hund i mange tilfeller gir en symptomatisk lindring av kroniske deformerende forandringer i ledd- og skjelettsystemet.

En er klar over at det i denne undersøkelsen hadde vært ønskelig med en større placebo/ cross-overgruppe. Men idet behandlingen synes å gi en såpass klar effekt, finner en det riktig å informere om dette på det nåværende tidspunkt. Undersøkelser med større placebogruppe pågår.

Undersøkelsen viser at poly-askorbat trolig er et godt alternativ ved behandling av denne type lidelser. Mange eldre hunder viser dessuten en generell bedret allmenntilstand ved behandling.

At askorbinsyre/askorbinsyremetabolitter i det hele tatt har virkning hos hund, en art som presumptivt skulle syntetisere tilstrekkelig askorbinsyre selv, kan peke i retning av at egenproduksjonen av askorbinsyre gir suboptimale konsentrasjoner i enkelte vev under visse forhold/lidelser.

Tabell 1.

| | Total antall | Antall hunder som viser: god bedring/ symptomfrihet | liten bedring/ ingen bedring |
|---|---|---|---|
| etter 1 uke | 100 | 75 | 25 |
| etter 6 uker | 100 | 79 | 21 |
| Hunder tilgjengelige etter > 6 mnd. | 85 | 65 | 20 |

Tabell 2.

| | Total antall | Antall hunder som viser: god bedring/ symptomfrihet | liten bedring/ ingen bedring |
|---|---|---|---|
| Halthet/smerte ved hofteleddsdysplasi: | | | |
| etter 1 uke | 45 | 32 | 13 |
| etter 6 uker | 45 | 35 | 10 |
| Spondylose prolaps i ryggsøyle | | | |
| etter 1 uke | 17 | 13 | 4 |
| etter >6 uker | 17 | 13 | 4 |
| Artroseforandringer | | | |
| etter 1 uke | 38 | 30 | 8 |
| etter >6 uker | 38 | 31 | 7 |

**Sammendrag:**
Undersøkelser utført ved Groruddalen dyreklinikk viser at polyaskorbat som er en blanding av askorbinsyre og askorbinsyremetabolitter, har en gunstig effekt på symptomer fra kroniske inflammasjoner i bevegelsesapparatet hos hund. Ca 75 % av de behandlede hundene viste bedring ved behandling. Videre undersøkelser er under arbeid.

**Summary**
POLYASCORBATE,(C-FLEX) AN ALTERNATIVE BY AILMENTS IN THE SUPPORT- AND MOVEMENT- STRUCTURE IN DOGS.
Clinical studies at Groruddalen Dyreklinikk have demonstrated that poly- ascorbate,a mixture of ascorbic acid and its metabolites has a positive effect on symptoms caused by chronic inflammations in dog's movement-structure. About75% of the dogs treated showed improvement upon treatment. Further investigations are in progress.

## REFERANSER

1. Levine M. New concepts in the biology and biochemistry of ascorbic acid. N Engl J Med 1986; 314: 898
2. Bush MJ. Verlangieri AJ. An acute study on the relative gastro- intestinal absorbtion of a novel form of calcum ascorbate. Res Commun Chem Pathol Pharmacol 1987; 57: 137- 40
3. Wright J. A clinical study of Polyascorbate vs L-ascorbic acid. Meridian Valley Clin Lab Report Kent, WA, USA.1987
4. Velangieri AJ. Relative Gastro-intestinal absorption of polyascorbate vs L-ascorbic acid. Atherosclerosis Res Lab Report, Univers, Mississipi 1988

**The Norwegian Veterinary Journal**, Volume 102, August/September 1990

**POLYASCORBATE (C-FLEX®), AN INTERESTING ALTERNATIVE BY PROBLEMS IN THE SUPPORT AND MOVEMENT APPARATUS IN DOGS**

*Clinical trial of ESTER-C® POLYASCORBATE in canines*

# Polyascorbate (C-Flex®), an interesting alternative by problems in the support and movement apparatus in dogs.

Geir Erik Berge
Groruddalen Dyreklinikk
Oslo, Norway

## Preliminary Discussion.

Ever since Dr. Lind in the year 1740 discovered that juice from citrus fruits could prevent scurvy in sailors, vitamin C has had a natural place in human nutrition. All species except primates, guinea pigs, certain types of bats, salmon varieties, certain insects and shellfish have enzyme systems that convert glucose to ascorbic acid (1). Vitamin C has hence received little attention within modern veterinary medicine. The assumption that animals at all times are capable of producing sufficient quantities themselves is however probably not a valid one.

Ascorbic acid plays a role in a large number of biochemical reactions in the metabolism of collagen and in the immune system as well as in a series of redox reactions.

Also metabolites of vitamin C are reactive components. Today's research gives these substances the attention they deserve. The conversion of ascorbic acid into these metabolites is believed to be dependent on the intra-cellular ascorbic acid concentration. Under certain circumstances, like infectious, traumatic, or physical stress, larger amounts of ascorbic acid and its metabolites are being consumed by various tissues.

Under these conditions, it is very well possible that the animal's own production cannot cope with the demand of supplying all tissues with optimal levels of ascorbic acid.

Ascorbic acid is an acidic, water soluble molecule which after ingestion is very rapidly excreted through the kidneys. Ascorbic acid has pka 4.17.

The ideal vitamin C would be a pH neutral molecule that would not cause irritation to the gastrointestinal tract, that is rapidly absorbed from the gut, that is more slowly excreted, and that has the ability to cross cell membranes in a more efficient way than does ascorbic acid, so that higher intra-cellular levels can be reached. Such a vitamin C (classified as a polyascorbate) has been developed and patented by Inter-Cal Corporation of Prescott, Arizona under the trademark C-Flex®.

Polyascorbate is a complex mixture of calcium ascorbate molecules and the above mentioned metabolites. In water solution the polyascorbate is pH neutral, which influences the osmolarity in the intestinal tract less than does ascorbic acid, which has a pH 2.4. It is absorbed faster than ascorbic acid in both animals and humans.

Furthermore, slower excretion and higher intra-cellular concentrations are achieved. Clinical studies suggest that the metabolites created during C-Flex's ® unique manufacturing process are of vital importance in its increased ability to penetrate cell membranes and thus give higher intra-cellular ascorbate absorption.

Robert Davis, Ph.D., at the Pennsylvania College of Podiatric Medicine observed that polyascorbate lessened both symptoms of pain and stiffness in arthritis patients (5). Indications of similar effects in dogs were found by Charles Dockter, DVM, in Prescott, Arizona (5). It is assumed that the metabolism in support, bone, and tissue is being stimulated and optimized.

The target for this study was to observe the effect of the polyascorbate in dogs with clinical symptoms of chronic inflammation processes in joints, skeleton and muscles, as routinely treated with antiphlogistics and corticoids.

## Materials and Methods.

The study was carried out at Groruddalen Dyreklinikk over a 6 month period in 1988. 100 dogs of different breeds and ages were given C-Flex ® approximately 30 mgs/kg bodyweight 3 times a day orally.

All treatments were given because owners saw the animal's symptoms of limping, lameness, limited movement ability and pain. Diagnosis was made on the bases of journals, clinical evaluation and, if necessary, X-ray. The effect was measured as changes in symptoms, partly by new clinical assessment and partly by owners reporting their evaluation of treatment. The effect was measured after 7-10 days, more than 6 weeks and after approximately 6 months.

A series of both acute and chronic ailments were treated. With acute problems and conditions that rapidly change, it is very difficult to distinguish between effect of treatment and other influential factors. Such patients were therefore excluded from the study. One has hence limited the study to observe effect of:

1) Symptoms that have a known cause and that are permanent, and
2) where symptoms had been stable over a minimum of 6 weeks, and
3) must be assumed to persist without treatment.

## TABLE 1

| Number of dogs showing | Total | Good improvement/ free of symptoms | Small improvement No improvement |
|---|---|---|---|
| After 1 week | 100 | 75 | 25 |
| After > 6 weeks | 100 | 79 | 21 |
| Available after > 6 months | 85 | 65 | 20 |

Splitting these numbers after diagnosis gives the following:

## TABLE 2

| | | | |
|---|---|---|---|
| Limping/pain from hip dysplasia: | | | |
| After 1 week | 45 | 32 | 13 |
| After > 6 weeks | 45 | 35 | 10 |
| Spondilosis back prolapse: | | | |
| After 1 week | 17 | 13 | 4 |
| After > 6 weeks | 17 | 13 | 4 |
| Arthrosis changes: | | | |
| After 1 week | 38 | 30 | 8 |
| After > 6 weeks | 38 | 31 | 7 |

Dogs with the following ailments were included:
Arthrosis
Spondylosis
Hip Dysplasia
Older disc prolapses with permanent secondary changes
Senile wear changes in support and movement tissue

## Results.

The results of the study are shown in table 1.

Results related to the different diagnosis are shown in table 2. No clear variations between the studied ailments were found.

There were no side effects seen during treatment.

C-Flex ® was administered both as tablets and powder. This did not seem to make a difference in effect.

In agreement with some owners, 6 dogs with chronic arthrosis were divided into 2 groups. Group 1 received C-Flex®, group 2 placebo. After 1 week all the dogs in group 1 showed improvement, while no one in group 2 did. Treatment was postponed for 4 weeks. Group 1 this time received placebo and group 2 received C-Flex®. After one week all the dogs in group 2 showed improvement and none in group 1 did. A second crossover was then made with the same results.

## Conclusion:

This study shows an interesting tendency that polyascorbate orally administered gives symptomatic relief of chronic deforming changes in the joint and skeleton system in many of these patients.

One realizes that it would have been preferable to have larger groups. However, as the treatment seemed to give such clear-cut effect, one deems it appropriate to mention this at this point. Further studies with larger groups are underway.

Polyascorbate should prove to be a good alternative to current treatment of these ailments. Many older dogs showed a general improvement during treatment. That the substance gives effect at all to dogs, a species that should be able to produce its own vitamin C, points in the direction that the dog's own production gives sub-optimal concentrations in some tissues under certain conditions.


## Summary:

A study on the clinical effect of C-Flex ® on chronic inflammation ailments in the support and movement structures in dogs is present. The mineral polyascorbate form contains significant levels of naturally occurring vitamin C metabolites. Ascorbic acid and its metabolites play important roles in approximately 300 metabolic reactions. Clinical studies at Groruddalen Dyreklinikk have demonstrated that polyascorbate has a positive effect on symptoms caused by chronic inflammation in the dog's movement structure. About 75% of the dogs treated showed improvement upon treatment. Further investigations are in progress.


## REFERENCES


1. Levine M. New concepts in the biology and biochemistry of ascorbic acid. N Engl J Med 1986; 314: 898
2. Bush MJ. Verlangieri AJ. An acute study on the relative gastro-intestinal absorption of a novel form of calcium ascorbate. Res Commun Chem Pathol Pharmacol 1987; 57: 137-40
3. Wright J. A clinical study of Polyascorbate vs L-ascorbic acid. Meridian Valley Clin Lab Report, Kent, WA, USA. 1987
4. Verlangieri AJ. Relative Gastro-intestinal absorption of polyascorbate vs L-ascorbic acid. Atherosclerosis Res Lab Report, Univers, Mississippi 1988

**INTER-CAL CORPORATION**
**533 Madison Avenue**
**Prescott, Arizona 86301 USA**

***(520) 445-8063 (voice)***
***(520) 778-7986 ( fax)***
***rsrch @ intercal.com (email)***

---

# *SAFETY OF ORAL VITAMIN C*

---

"Many persons habitually ingest 1 g or more of ascorbic acid without developing apparent toxic manifestations."

**Subcommittee on the Tenth Edition of the RDAs. 1989. Recommended Dietary Allowances. 10th Edition. Food and Nutrition Board, Commission on Life Sciences, National Research Council. Washington DC: National Academy Press. ISBN 0-309-04041-8.**

"Megadoses of ascorbic acid have been reported to have beneficial effects on resistance to the common cold and various diseases. ... For most individuals ascorbic acid has a low toxicity and, hence, excessive intakes are tolerated. Adverse effects of high ascorbate supplements have been reported on occasion, such as hypoglycemia, induced uricosuria, dependency or rebound effect, and hemolysis in patients with erythrocyte glucose-6-phosphate dehydrogenase deficiency.

"With excessive intake the absorbed ascorbic acid is largely excreted into the urine. Only a small amount is metabolized to oxalate regardless of the level of intake. However, studies indicate that individuals who form kidney stones should avoid the intake of high doses of Vitamin C. This also is true for patients with renal impairment or who are on chronic hemodialysis."

**Sauberlich, Howerde E. 1990. Chapter 15. Ascorbic Acid. In: Present Knowledge in Nutrition, 6th edition, Myrtle L. Brown, editor. Washington DC: International Life Sciences Institute, Nutrition Foundation. pp. 132-141. ISBN 0-944398-05-7.**

"Vitamin C is a remarkably innocuous substance, one of the least toxic substances known. Studies with guinea pigs showed that they could be given doses of 5 g per kilogram body weight per day orally or by subcutaneous or intravenous injection without developing symptoms of toxicity [Demole, V. 1934. On the physiological action of ascorbic acid and some related compounds. ***Biochemical Journal 28***: 770-773], and similar observations have been made with mice, rabbits, cats, and dogs. By extrapolation this represents a daily dose of 350 g in the adult human.

"Amounts of 200 g have been taken in one day by mouth by humans without serious consequences, and 100 g or more of sodium ascorbate have been given by intravenous infusion to patients with benefit rather than harm. Hundreds of people have taken 10 to 20 g per day over periods of years or decades with no indication of harmful long-term side-effects. The fact that animals of most species synthesize corresponding amounts makes this degree of tolerance of the substance reasonable.

"There has been much discussion in newspaper articles about the possibility that a high intake of vitamin C may cause the development of kidney stones. This suggestion is largely based on a misunderstanding. It is known that some kinds of kidney stones (the less common ones) tend to form in acidic urine, and others tend to form in alkaline urine. Physicians sometimes recommend to certain patients that they keep their urine either alkaline or acidic, in case that it is known that the patient may develop kidney stones of one kind or the other. Large doses of vitamin C as sodium ascorbate keep the urine alkaline, whereas large doses of this vitamin as ascorbic acid make it acidic. This question really has nothing to do with vitamin C, because any other alkalizing agent, such as potassium citrate, can be used to make the urine alkaline, and any acidifier, such as ammonium chloride, can be used to make it acidic. Some people, of rare genotypes, such as those who convert most of the ascorbate to oxalate, may be unable to tolerate large doses of vitamin C, but the very small number of the cases reported in the medical literature indicates that there are not very many of these unfortunate individuals. The risk of kidney stone formation as a result of high ascorbate intake is very remote indeed.

"A few years ago many people were disturbed and some of them stopped taking supplementary vitamin C when a report was published and widely discussed in newspapers that a high intake of vitamin C taken with a meal might destroy as much as 95 percent of the vitamin B12 contained in the food, possibly leading to a serious disease resembling pernicious anemia. Later careful studies by two groups of investigators showed that this report was erroneous, largely because a poor method of analysis for vitamin B12 had been used. The form of vitamin B12 found in foods, hydroxycobalamin, is to some extent susceptible to attack by vitamin C.

In the stomachs of persons other than those with pernicious anemia, however, the B12 combines with intrinsic factor to form a complex that can be transported into the blood stream. This complex is stable: it is not destroyed by vitamin C. Moreover, the form of vitamin B12 that is present in vitamin tablets is cyanocobalamin, which is not destroyed by vitamin C. The concern about vitamin C in relation to vitamin B 12 was accordingly not based on solid facts.

"The most common side-effect of large doses of vitamin C is looseness of the bowels, which in some people may be described as a mild temporary diarrhea, and an increased tendency to produce intestinal gas. A few grams of vitamin C taken without other food may serve as an excellent laxative.... Some people tolerate sodium ascorbate better than ascorbic acid, and tablets, including timed-release tablets, better than the pure crystalline vitamin. Most people can take 15 or 20 g per day, in divided doses (3 or more per day) without much gastrointestinal distress, and sick people can take much larger amounts."

**Cameron, E., and L. Pauling. 1979. Cancer and Vitamin C. A Discussion of the Nature, Causes, Prevention, and Treatment of Cancer with Special Reference to the Value of Vitamin C. Menlo Park, CA: Linus Pauling Institute of Science and Medicine. pp 118-119. ISBN 0-393-50000-4.**

"For the record, we have summarized the literature and our own experiences in two articles [Ringsdorf, W. M., and E. Cheraskin. 1984. Medical complications from ascorbic acid: a review and interpretation (Part One). ***Journal of Holistic Medicine 6*** (#1, Spring/Summer): 49-63; Ringsdorf, W. M., and E. Cheraskin. 1984. Medical complications from ascorbic acid: a review and interpretation (Part Two). ***Journal of Holistic Medicine 6*** (#2, Fall/Winter): 173-183]. This has also been released in a full chapter entitled 'Can Vitamin C Hurt You?' [Cheraskin, E., W. M. Ringsdorf, and E. L. Sisley. 1983. ***The Vitamin C Connection***. New York, NY: Harper and Row Publishers, Inc. (hardback), pp. 201-219; [Cheraskin, E., W. M. Ringsdorf, and E. L. Sisley. 1984. ***The Vitamin C Connection***. New York, NY: Bantam Books, Inc. (paperback), pp. 221-240]. But the most recent and complete summarization is available in a supplement of the International Journal for Vitamin and Nutrition Research [Rivers, J. M. 1989. Safety of high-level Vitamin C ingestion. ***International Journal of Vitamin and Nutritional Research (supplement) 30***: 95-102]. And is well stated in the following:

> 'Despite contradictory reports the concensus from an extensive literature search is that these adverse health effects are not induced in healthy persons by ingesting large doses of ascorbic acid.'

"Of all the alleged clinical contraindications to megavitamin C, the only one, more a common inconvenience, is diarrhea. Taking too much C (whatever the amount may be) may cause loose stools."

**Cheraskin, E. 1993. Chapter 15. How Much Vitamin C Should I Take? In: Vitamin C: Who Needs It? Birmingham, AL: Arlington Press. ISBN 0-944353-04-5.**

"An attempt has been made in this review to select papers that represent opposing views and to present a critical nonbiased interpretation of the results. This has led to the conclusion that the practice of ingesting large quantities of ascorbic acid [5 grams or more] will not result in calcium-oxalate stones, increased uric acid excretion, impaired vitamin B12 status, iron overload, systemic conditioning, or increased mutagenic activity in healthy individuals."

**Rivers, J. M. 1987. Safety of high-level Vitamin C ingestion. In: Third Conference on Vitamin C. Burns, J. J., J. M. Rivers, and L. J. Machlin, eds. Annals of the New York Academy of Sciences *498*: 445-454.**

"A comprehensive review of the literature indicates that populations with long-term consumption of higher than RDA levels of vitamin C (greater than or equal to 60 mg/day) from foods and/or supplements have reduced risks of cancer at several sites, cardiovascular disease, and cataracts. The safety of higher than RDA intakes of vitamin C is confirmed in eight placebo-controlled, double-blind studies and six non-placebo clinical trials in which up to 10,000 mg of vitamin C was consumed daily for up to 3 years. There are no clinical data which suggest that vitamin C's enhancement of non-heme iron absorption in individuals with low iron status could be a critical factor in the possible increased risk of heterozygous hemochromatosis-related cardiovascular disease. In fact, the cumulative data do not confirm that iron status is related to risk of cardiovascular disease. Moreover, higher than RDA intakes of vitamin C have been associated with several indices of lowered cardiovascular disease risk including increases in HDL, and decreases in LDL oxidation, blood pressure, and cardiovascular mortality."

**Bendich, A., and L. Langseth. 1995. The health effects of vitamin C supplementation: a review. Journal of the American College of Nutrition *14*: 124-136.**

"The few literature references suggesting adverse effects of high doses of ascorbic acid are outnumbered by a large number of clinical studies in which no adverse effects have been observed. Up to 5 g ascorbic acid daily may be administered safely even over a long term. Favourable effects of even higher doses in man may justify therapeutic trials in the range of 15 g daily which in our trials have proven safe during treatment of up to 2 years."

**Hanck, A. 1982. Tolerance and effects of high doses of ascorbic acid. Dosis facit venenum. Int. J. Vitam. Nutr. Res. *Suppl. 23*: 221-238.**

"Epidemiologic evidence links high antioxidant status with low risk of degenerative disease. Optimal intakes of antioxidants may not be achievable by diet alone; supplements may be taken, particularly in subgroups of the population at high risk. ... Massive anecdotal evidence exists that vitamin C (at greater than or equal to 1 g/d) is safe. Exhaustive literature searches have failed to reveal a controlled study of vitamin C toxicity in human subjects. Anxiety exists about oxalate stone formation, uricosuria, vitamin B-12 destruction, mutagenicity, and iron overload, but the consensus is that adverse effects do not occur in healthy subjects ingesting large amounts of vitamin C."

**Diplock, A. T. 1995. Safety of antioxidant vitamins and beta-carotene. American Journal of Clinical Nutrition. *Supplement* 62: S1510-S1516.**

Life Sciences, Vol. 49, pp. 1377-1381
Printed in the U.S.A.

Pergamon Press

# STIMULATORY ACTION OF CALCIUM L-THREONATE ON ASCORBIC ACID UPTAKE BY A HUMAN T-LYMPHOMA CELL LINE

Michael J. Fay and Anthony J. Verlangieri[1]

Department of Pharmacology, University of Mississippi
School of Pharmacy, University, MS 38677

(Received in final form August 28, 1991)

## Summary

The effects of preincubation of human T-lymphoma cells with increasing concentrations of calcium L-threonate on the uptake of L-[1-$^{14}$C]ascorbic acid were examined. Calcium L-threonate (0 - 1,000 mg%) stimulated ascorbic acid (1.25 mg%) uptake in a dose-dependent manner. These results indicate that calcium threonate and possibly other ascorbic acid metabolites have biological activity and potential pharmacological applications.

The metabolism of ascorbic acid involves the oxidation of ascorbic acid to dehydroascorbic acid which subsequently undergoes a hydrolytic ring cleavage to form 2,3 diketo-L-gulonic acid. Diketo-L-gulonic acid may become decarboxylated to form L-lyxonic acid, L-xylose, and L-xylonic acid. Two other metabolic products resulting from further oxidation of 2,3 diketo-L-gulonic acid are L-threonic acid and oxalic acid (1).

There is little information concerning the biological, toxicological and possible pharmacological actions of L-threonic acid and its salts, as well as the other metabolites of ascorbic acid. Thomas and Hughes speculated that threonic acid does not interfere with the gastrointestinal absorption of ascorbic acid in guinea pigs (2). One study showed that excessive amounts of threonic acid administered to rats (120 days) and mice had no significant effects on growth rates, organ weights, biochemical parameters and survival times as compared to controls (3). Ester-C® (U.S. patent number 4,822,816, InterCal Corp. Prescott, AZ) is a form of calcium ascorbate that contains ascorbic acid metabolites produced in the manufacturing process. The specifications for Ester-C® are as follows: ascorbic acid as calcium ascorbate (78% - 84%), ascorbate activity (70% - 75%), dehydroascorbic acid (5% - 9%), calcium oxalate (< 0.4%), calcium threonate (< 3%), calcium

---

[1] To whom all correspondence should be sent.

0024-3205/91 $3.00 + .00
Copyright © 1991 Pergamon Press plc

carbonate (< 4.5%), and free moisture (< 2%). The name Ester-C® is simply a trademark and is not meant to imply a chemical ester. A study by Bush and Verlangieri indicated that Ester-C® is absorbed more readily and excreted less rapidly than L-ascorbic acid in rats (4). Experiments in dogs indicated that Ester-C® has positive therapeutic effects on chronic bone inflammation and arthritis. The author suggests that the presence of ascorbic acid metabolites in Ester-C® may be partly responsible (5).

The objective of the present study was to investigate the effect of calcium L-threonate, the major calcium aldonate found in Ester-C®, on the uptake of L-[1-$^{14}$C]ascorbic acid by a human T-lymphoma cell line.

## Materials and Methods

H9 cells cloned from a specific HUT 78 human cutaneous T cell lymphoma (ATCC TIB 161) were the gift of Dr. S. Harakeh from the Linus Pauling Institute (6,7,8,9). Cells were grown in RPMI-1640 medium (Sigma) supplemented with 10% fetal bovine serum (Gibco) and 50$\mu$g/ml Gentamicin (Gibco). Cells were subcultured every 2 - 3 days by centrifugation (100xG, 10 min) and resuspended in complete growth medium at a concentration of $10^5$ cells/ml. Two days prior to experimentation, cells were subcultured as stated above. Approximately twenty hours before experimentation cells were transferred to serum free RPMI-1640 medium containing 0.1% bovine serum albumin (Sigma). In the present experiments, cells were serum starved for 20 hours to deplete levels of serum proteins such as insulin, which has been shown to stimulate cellular ascorbic acid uptake (10). Cells were centrifuged as previously described and the pellet was washed with Ringer's salt solution (pH 7.4). This was repeated once. Washed cells were suspended in Ringer's and counted using the trypan blue exclusion method to determine viability. Cells were seeded into 15 ml sterile centrifuge tubes (Corning) at a density of $10^6$ cells/tube and centrifuged as above. The cell pellets were suspended in 1.0 ml of calcium L-threonate of varying concentrations (0 - 1,000 mg%) prepared with sterile Ringer's (pH 7.4). Calcium L-threonate (Pliva Chemicals, Yugoslavia) was prepared using a modified method of oxidizing ascorbic acid with hydrogen peroxide (11) developed by Dr. Seth Rose, Dept. of Chemistry, Arizona State University (Tempe, AZ). All tubes were incubated for 1 hour at 37° C in a 5% $CO_2$/95% air humidified incubator. After 1 hour all tubes received 1.0 ml of 2.5 mg% unlabelled ascorbic acid (Sigma) to which 0.125 $\mu$Ci/tube L-[1-$^{14}$C]ascorbic acid was added (NEN/Dupont, specific activity 9.90 mCi/mmol), giving a final ascorbic acid concentration of approximately 1.25 mg%. After 30 minutes the uptake was terminated by rapid addition of 4.0 ml of ice cold Ringer's to each tube. Cells were pelleted as previously described and washed with 2.0 ml of ice cold Ringer's. This was repeated twice. Lymphoma cells were then solubilized with 1 N NaOH for 2 hours at room temperature. A 0.25 ml aliquot of the digested sample was added to 0.25 ml of 1 N HCl for neutralization and this was added to 10 ml of Instagel® (Packard). After dark adaptation, samples were counted 3 times for 5 minutes using a Packard 3255 liquid scintillation counter. Counts were converted from cpm to dpm using a quench correction factor.

Differences among groups were tested at $P < 0.05$ by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (MRT), using the SPSS-x statistical software package (SPSS Inc., 444 N Michigan Ave., Chicago, IL 60611).

## Results

The data for cellular uptake of L-[1-$^{14}$C]ascorbic acid are expressed as DPM/$10^6$ cells and as percent increase from control (0 mg% calcium threonate). The results in Table I clearly show that calcium L-threonate increases cellular ascorbic acid accumulation, with the highest dose of calcium L-threonate increasing uptake 177% over control values.

TABLE I

THE EFFECT OF CALCIUM L-THREONATE ON L-[1-$^{14}$C]ASCORBIC ACID UPTAKE BY HUMAN T LYMPHOMA CELLS

| GROUP[1] | DPM/$10^6$ CELLS[2] | % INCREASE |
|---|---|---|
| 0 mg%(CONTROL) | 1,662 ± 83 | 0 % |
| 100 mg% | 2,302 ± 199 | 39 % |
| 500 mg% | 2,915 ± 130[a] | 75 % |
| 750 mg% | 4,177 ± 567[a,b,c] | 151 % |
| 1,000 mg% | 4,610 ± 597[a,b,c] | 177 % |

1 5 samples of $10^6$ cells per group
2 Mean DPM/$10^6$ cells ± standard error of the mean
a Statistically significant from control ($p < 0.05$)
b Statistically significant from 100 mg% ($p < 0.05$)
c Statistically significant from 500 mg% ($p < 0.05$)

## Discussion

In addition to its role in the prevention of scurvy, ascorbic acid has been implicated in immune cell function (12,13), cholesterol metabolism (14), the prevention of cardiovascular disease (15), and also in cancer treatment and/or prevention (16,17). More recently, ascorbic acid has been shown to suppress the replication of the human immunodeficiency virus (HIV) in infected human T-lymphoma cells as measured by a reduction in extracellular reverse transcriptase and p24 antigen (18). Although much work has been done on the biological and pharmacological effects of ascorbic acid, very little work has been done with the metabolites of ascorbic acid degradation.

The present work clearly shows that calcium L-threonate stimulates the uptake of ascorbic acid in a dose-dependent manner

by human T-lymphoma cells. These results may explain why Ester-C®, a calcium ascorbate supplement containing ascorbic acid metabolites, is more readily absorbed than ascorbic acid (4). We conducted a similar T-lymphoma uptake study using L-tartaric acid (Sigma) at a concentration equivalent to the threonate present in the 1,000 mg% calcium L-threonate treatment group. Tartaric acid is a four carbon aldonic acid that is not an ascorbic acid metabolite. The results of this study indicates that L-tartaric acid does not increase ascorbic acid uptake (unpublished data), and suggests a specific effect of calcium L-threonate.

The results of the present study suggest that calcium threonate in combination with ascorbic acid or Ester-C® alone may be useful in the management of the HIV positive patient. This may include a synergistic combination of the above which may increase the inhibition of HIV by AZT alone. This combination may result in a lower effective AZT dose with a reduction of AZT side-effects. Increased cellular uptake of ascorbic acid by calcium threonate may have an impact on the treatment and/or prevention of cardiovascular disease, some types of cancer, AIDS, as well as increase cellular free-radical scavenging. Future work will be directed at elucidating the mechanism by which calcium threonate stimulates cellular ascorbic acid uptake.

## Acknowledgments

This work was supported in part by overhead funds of Anthony J. Verlangieri, Ph.D., and by a fellowship awarded to Michael J. Fay from the InterCal Corp. (Prescott, AZ).

## References

1. M. LEVINE and M. KYOJI, Vitam. Horm. 42 1-64 (1985).
2. M. THOMAS and R.E. HUGHES, Fd. Chem. Toxic. 21 449-452 (1983).
3. M. THOMAS and R.E.HUGHES, Fd. Chem. 17 79-83 (1985).
4. M.J. BUSH and A.J. VERLANGIERI, Res. Commun. Chem. Path. Pharmacol. 57 137-140 (1987).
5. G.E. Berge, Nor. Vet. J. 102 579-581 (1990).
6. M. POPOVIC, E. READ-CONNOLE and R.C. GALLO, Lancet 22(29) 1472-1473 (1984).
7. D.L. MANN, S.J. O'BRIEN, D.A. GILBERT, Y. REID, M. POPOVIC, E. READ-CONNOLE, R. GALLO and A. GAZDAR, Aids Res. Human Retro. 5 253-255 (1989).
8. A.F. GAZDAR, D.N. CARNEY, P.A. BUNN, E.F. RUSSELL, E.S. JAFFE, G.P. SCHECTOR and J.G. GUCCION, Blood 55 409-417 (1980).
9. J.E. GOOTENBERG, F.W. RUSCETTI, J.W. MIER, A. GAZDAR and R.C. GALLO, J. Exp. Med. 154 1403-1418 (1981).
10. A.J. VERLANGIERI and J. SESTITO, Life Sci. 29 5-9 (1981).
11. H.S. ISBELL and H.L. FRUSH, Carb. Res. 72 301-304 (1979).
12. R. ANDERSON, Vitamin C: Ascorbic acid, J.N. COUNSELL and D.H. HORNIG (eds.) pp. 249-72, Applied Science Publishers, London (1981).
13. W. PRINZ, R. BORTZ, B. BREGIN and M. HERSCH, Int. J. Vitam. Nutr. Res. 47 248-257 (1977).
14. E. GINTER, Science 179 702-704 (1973).

15. A.J. VERLANGIERI, J.C. KAPEGHIAN, S. EL-DEAN and M.J. BUSH, Med. Hypoth. 16 7-15 (1985).
16. E. CAMERON and L. PAULING, Proc. Natl. Acad. Sci. USA 73 3685-3689 (1976).
17. R.E. WITTES, N. Engl. J. Med. 312 178-179 (1986).
18. S. HARAKEH, R.J. JARIWALLA and L.PAULING, Proc. Natl. Acad. Sci. USA 87 7245-7249 (1990).

# Vitamin C—a conditionally essential nutrient for dogs, cats and horses

Do "complete and balanced" pet foods really provide all the nutrients and, most importantly, the proper levels required for health and vitality? Can a 26 week feeding trial, as prescribed by the American Association of Feed Control Officials (AAFCO), produce enough statistically relevant data to determine if a food fulfills the lifetime nutrient needs of dogs and cats? And, finally, to what extent do stress, age, pregnancy and exposure to chemicals and infectious agents change the nutrient needs of animals? Veterinary nutritionists and clinicians are intensifying their search into the relationship between altered physiological states and macro/micro nutrients, and are better defining "essential" and "conditionally essential" nutrients.

Essential nutrients are necessary for maintaining life and must be provided in the food, since they cannot be synthesized in the body. A chronic deficiency of the amino acid taurine in cats, for example, results in dilated cardiomyopathy, central retinal degeneration and blindness. Without supplemental vitamin C, guinea pigs develop scurvy and eventually die. Other nutrients, however, may become essential only when the normal homeostatic state is upset. Obese "conditionally essential" nutrients can be supplied exogenously or produced endogenously. Vitamin C is a potent antioxidant and is considered by many to be a prime example of a conditionally essential nutrient in dogs, cats and horses.

Vitamin C has excellent antioxidant properties, is an essential component of many cellular biochemical reactions and may protect against free radical-mediated tissue damage. Although dogs, cats and horses (unlike humans and other primates, guinea pigs, fruit bats and red-vented bulbul birds) are capable of producing their own vitamin C, it is uncertain whether the effects produced are enough to counter the effects of stress, disease, exercise, breed predilections to inherited dysfunctions, environmental pollutants and irritants, poor quality foods and age.

Last year the Inter-Cal Corporation of Prescott, Arizona, began providing Best Friends Ester-C, a non-acidic product containing vitamin C, calcium ascorbate and C metabolites. Ester-C helped many of the dogs walk and "feel" better. In fact, in a series of double-blind crossover trials, Ester-C proved twice as effective as ordinary vitamin C in reducing lameness and discomfort and in increasing mobility and activity. Our work confirmed the results of earlier studies by a Norwegian veterinarian who had found that Ester-C improved the mobility of dogs suffering from various joint disorders.

Supplementing the diet of dogs, cats and horses with vitamin C may be a way to reduce free radical damage to tissue and organ systems and prevent, or even reverse, disease. Unfortunately, ordinary vitamin C is an acid and many animals are unable to tolerate the dose necessary to achieve maximum intracellular levels. From our experience at Best Friends, it appears that Ester-C is the best way to provide this important conditionally essential nutrient to pets.

**THE USE OF ESTER-C® FOR JOINT DISCOMFORT IN DOGS - A STUDY**

**L. Phillips Brown, DVM**

## Preliminary Discussion

Vitamin C (ascorbic acid) is vital for maintaining the physical integrity of collagen. Cartilage consists of collagen, proteoglyciens and water. Collagen is a tough, stringy protein that physically connects tissues and provides the tensile strengthening cartilage. Proteoglycions trap and hold water, allowing for compression resistance. In normal cartilage, the anabolic and catabolic processes of chemdrocyte cells are in qualibrium. In degenerative (ie.. aging) or inflammatory conditions, the processes are excessive.

Because humans (and other species, such as guinea pigs) cannot manufacture endogenous ascorbic acid, vitamin C must be in their daily diet. In fact, millions of people routinely supplement their diets with vitamin C in one form or another. Dogs, on the other hand, can manufacture ascorbic acid and should not require additional vitamin C.

Earlier studies in dogs by Belfield and Kronfeld and later by Berge and Docktor, demonstrated that "extra" vitamin C might be beneficial in reducing chronic inflammation.

Unfortunately, many animals cannot tolerate the levels of ordinary vitamin C needed to provide beneficial results. Dogs may not manufacture sufficient quantities of ascorbic acid to counter the effects of aging, stress, breed predilections to inherited dysfunctions, environmental irritants and pollutants and poor quality of high fat pet foods. A form of vitamin C that would provide higher levels of intracellular ascorbic acid without negative side effects would-be a useful and unique product.

## Product Background

According to the manufacturer, Inter-Cal Corporation, of Prescott, Arizona, Ester-C® is a patented ascorbate supplement that contains calcium ascorbate, naturally occurring dehydroascorbate and the vitamin C metabolite, threonate. Threonate permits ascorbate to be more rapidly absorbed from the gastrointestinal tract, cross cell membranes more efficiently, reach higher cellular levels and be excreted more slowly than ordinary vitamin C. Ester-C® ascorbate is pH neutral and believed to be water and partially fat soluble.

## Mechanism of Action

Vitamin C may act as an immunoresponsive and chrondrogenerative agent. In degenerative (i.e., aging) or inflammatory conditions, collagen breakdown is excessive, resulting in joint discomfort and skeletal changes. A product that would provide high and prolonged levels of ascorbic acid could help increase the compression resistance of cartilage, mobilize white blood cells to the site of inflammation and enhance prostaglandin synthesis. The net result would be increased comfort and mobility.

## Clinical Studies

Five groups of dogs were given ascorbic acid in various forms and mineral combinations. (see table 1) orally twice daily according to the schedule listed in table 2.

| Compound Code | Composition |
|---|---|
| A101 | 2000 mg vitamin C from Ester-C® calcium ascorbate/tsp |
| A102 | 850 mg vitamin C from Ester-C® calcium ascorbate/tsp |
| A103 | 2000 mg vitamin C from Ester-C® Ca/Mg/K/Zn ascorbate/tsp |
| A104 | 2000 mg vitamin C from ascorbic acid/tsp |
| A105 | Microcrystalline cellulose |

**Table 1**

| Group | Phase I | Phase II | Phase III |
|---|---|---|---|
| I | A101 | A105 | A101 |
| II | A102 | A104 | A101 |
| III | A103 | A101 | A104 |
| IV | A104 | A102 | A101 |
| V | A105 | A101 | A105 |

**Table 2**

The response to each compound was measured by changes in comfort and activity levels and quantifies as follows:

| Grade | Response |
|---|---|
| 0 | No Change |
| 1 | Slight/Mild Improvement |
| 2 | Moderate/Good Improvement |
| 3 | Very Good Mobility |

**Table 3**

## Results

(See table 4B & 4C - Attached)

## Conclusion

The findings suggest that although dogs can manufacture endogenous ascorbic acid, the amount produced may not be sufficient to prevent or counteract stresses associated with injury or joint malpositioning. Low or declining ascorbic acid availability within the chondrocytes may compromise collagen homeostasis, resulting in a loss of tissue elasticity and compression resistance.

Clinical signs of stiffness, discomfort or decreased mobility may be reversed by high levels of ascorbic acid.

The consistency and degree of response to Ester-C® calcium ascorbate (Inter-Cal Corporation) suggests that it may be more beneficial in treating nonspecific locomotor discomfort than ordinary vitamin C, without signs of gastrointestinal irritation. The deterioration of "improved dogs" upon discontinuance of Ester-C® further verified it's usefulness as primary or adjunctive therapy.

From the results of this study, there is sufficient evidence to recommend oral Ester-C® calcium ascorbate to alleviate the pain and discomfort associated with certain musculoskeletal dysfunctions of dogs.

## Owner Comments After Use Of Ester-C® (C-Flex® Ascorbate)

Betsy Stix: "Tahoe...marked change in behavior...climbed stairs with comparative ease...eager to run...more playful...more energy."

Anne Mejia: "Lucky...marked difference...doesn't hesitate to join in on the activities...doesn't limp at all...my 'Happy-go-lucky' guy once again."

Diana Castle: "Tippy...gradually began using the leg and eventually had full use of it."

Dr. Richard Allen: "Mitzie...more active...able to walk up steps..walk 'up on her toes' again...no other prescription drugs before or during."

**Note**: The average extend of mobility improvement was calculated by adding total numerical value of response (Table 3) divided by the total number of animals responding to each compound.

## Biography

Dr. Brown is a graduate of the School of Veterinary Medicine of the University of California at Davis. A small animal clinician for over twenty years, Dr. Brown currently consults for several companies that manufacture and market natural pet products for companion animals. For further information on the clinical studies, Dr. Brown can be contacted at P.O. Box 1177, St. Augustine, FL 32085.

## References


Adkins, T.O. and Kronfeld, D.S.: Diet of racing and sled dogs affects erythrocyte depression by stress. Canadian Vet. J., 23:260-3, 1982

Allen, T.A. and Hands, M.S.: Conditionally Essential Nutrients. Proc. 8th ACVIM Forum, pp. 809-811, 1990

Belfield, W.O.: Chronic Subclinical Scurvy and Canine Hip Dysplasia. Veterinary Medicine/Small Animal Clinician. 1399-1401, 1976

Berge, G.E.: Polyascorbate (C-Flex), an interesting alternative by problems in support and movement apparatus in dogs. Norwegian Veterinary Journal. 102:579-581, 1990.

Docktor, C.: unpublished data.

Donoghue, S., Kronfeld, D.S., and Banta, C.A.: A possible vitamin C requirements in racing sled dogs fed a high fat diet. In: Nutrition, **Malnutrition and Diet in Dogs and Cats** ed. by Meyer, H. and Kienzle, E. pp. 110-114, Hanover: Tierarztliche Hochschule, 1988.

Fay, M.J.: Possible effects of ascorbic acid metabolites on the cellular uptake of ascorbic acid and other compounds. A dissertation for degree of Doctor of Philosophy, University of Mississippi, 1992.

Goodman, S.., **Vitamin C: The Master Nutrient**, Keats Publishing, Inc., Connecticut, 1991.

Kronfeld, D.S.: Stress supplements: Protein and vitamin C. Purebred Dogs/Kennel Gazette, 100.
10:8-9, 1983.

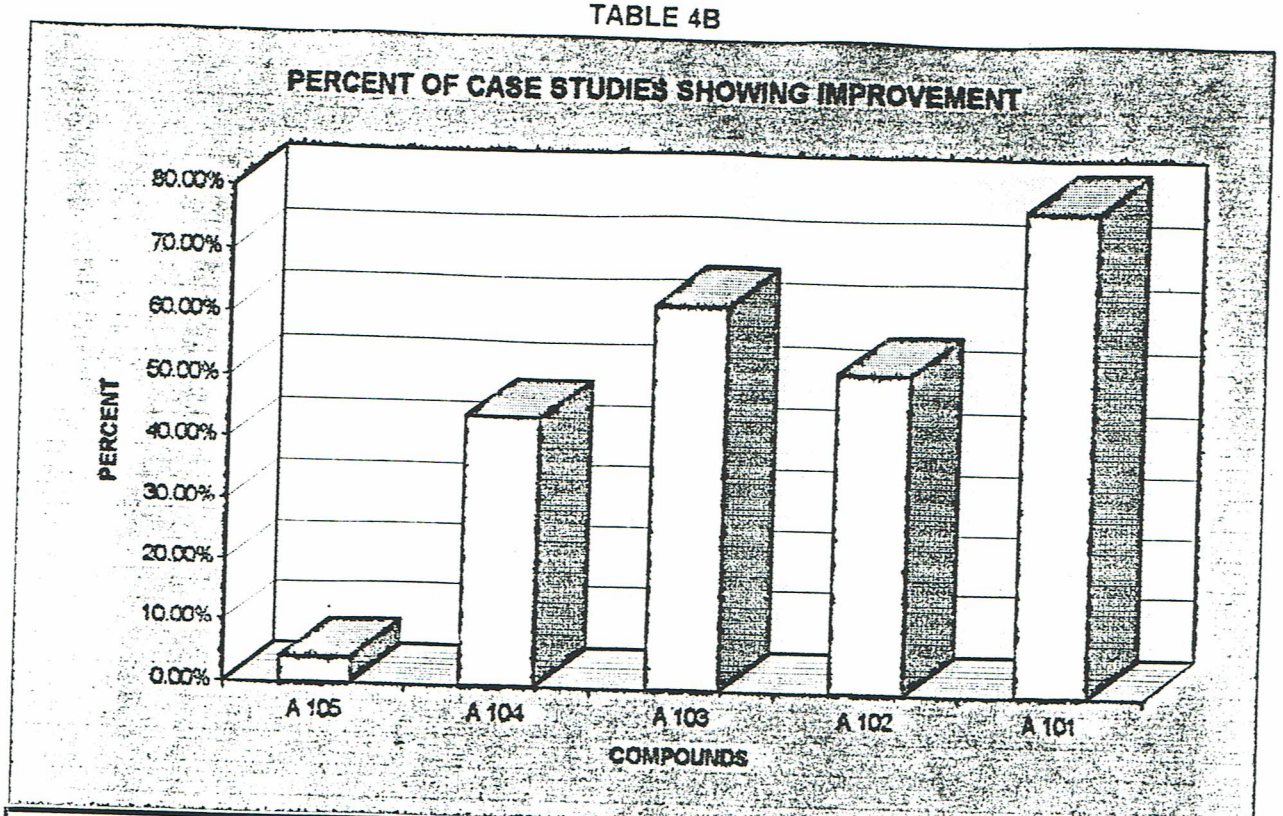
Lewis, D.L., Morris, Jr. M.L., and Hand, M.S.,: **Small Animal Clinical Nutrition** Mark Morris Assoc:, Kansas, 1990.

Newman, N.L., unpublished data.

Smith, C.: More on vitamin C. Natural Pet. Vol 2, No. 6:18-19, 1993

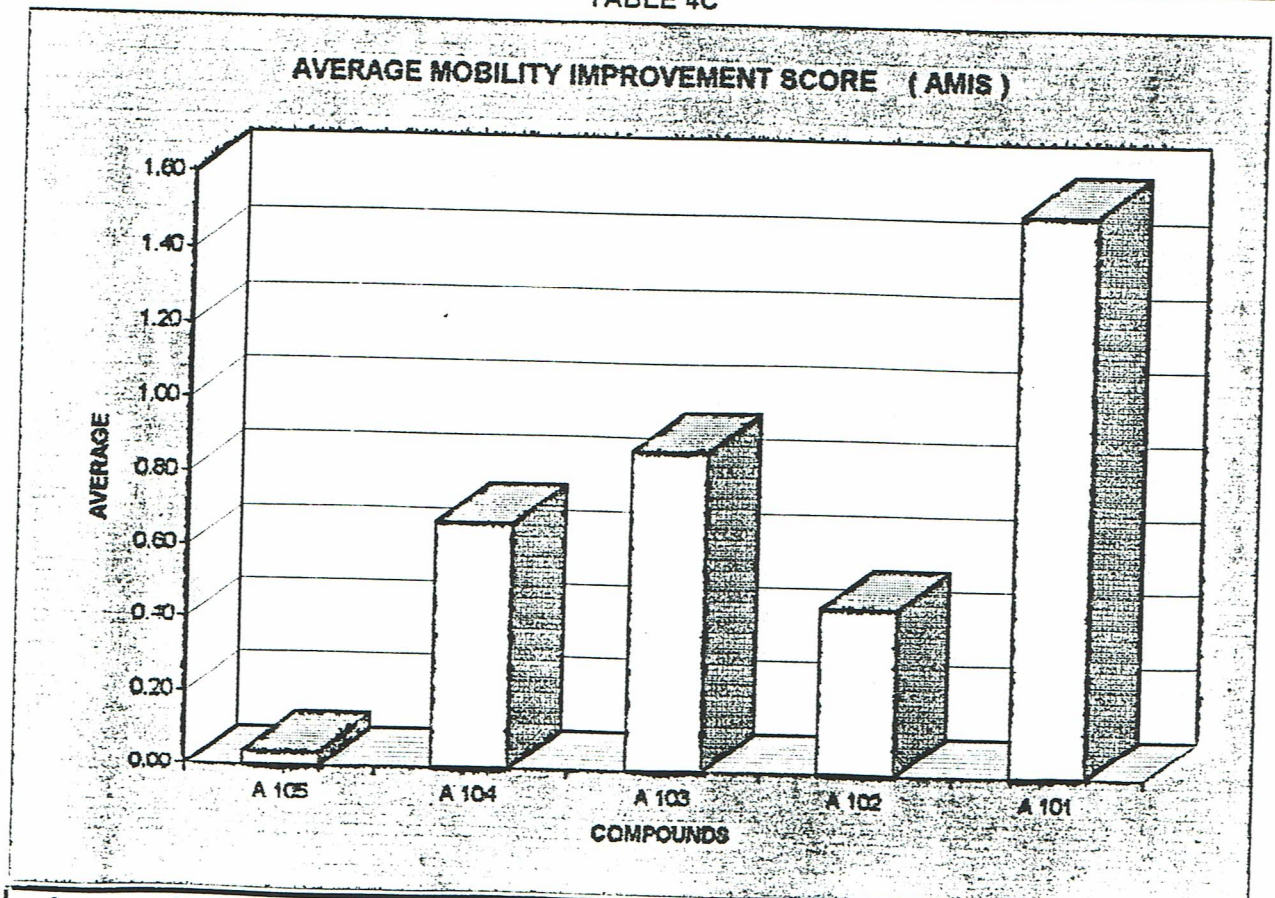
Stein, D. **Natural Healing for Dogs and Cats** The Crossing Press, California, 1993.

TABLE 4B




| Percentage Mobility Improvement Score | A 105 | A 104 | A 103 | A 102 | A 101 |
|---|---|---|---|---|---|
| | 4.00% | 44.00% | 62.00% | 52.00% | 78.00% |

TABLE 4C




| Average Mobility Improvement Score | A 105 | A 104 | A 103 | A 102 | A 101 |
|---|---|---|---|---|---|
| | 0.04 | 0.67 | 0.87 | 0.45 | 1.52 |

# INTER-CAL CORPORATION

**533 Madison Avenue**
**Prescott, Arizona 86301 USA**

*(520) 445-8063 (voice)*
*(520) 778-7986 (fax)*
*intercal@bslnet.com (email)*


---

## OVERVIEW OF ESTER-C®
## AND
## ESTER-C® RESEARCH

The profound biological effects of high doses of Vitamin C have led many researchers and health professionals to appreciate that large doses of Vitamin C behave differently in the body than smaller, nutritional doses. Convincing arguments have recently been presented for raising the dietary intake of Vitamin C above present recommended levels to amounts that promote optimal function of the dozens of biochemical reactions in which ascorbate is known to participate as an essential co-factor (Levine and Hartzell, 1987).

### Vitamin C Metabolites

The body changes Vitamin C into numerous metabolites that may have physiological actions that are different from those of Vitamin C itself. And these metabolites -- whether manufactured in the body or ingested along with supplemental Vitamin C -- may influence how the vitamin itself is transported and utilized. Dehydroascorbate -- the oxidized and transportable form of ascorbate -- may be "inactivated" by hydrolysis (i.e., delactonization) to 2,3-diketogulonate (Schmidt, Oberritter, Bruchelt, Hagmaier & Hornig, 1983). Dehydroascorbate, via the diketogulonate intermediate, may also be degraded both enzymatically and non-enzymatically in many tissues into smaller fragments, such as oxalate and aldonic acids (Kagawa & Takiguchi, 1962). The level of these metabolites in the body may not rise significantly, however, until very large doses of Vitamin C are administered.

Both the reduced and oxidized forms of ascorbate possess Vitamin C activity, because both forms are interchangeable in the body. They are metabolized quite differently in biological systems, however. Compared to the reduced ascorbate, the oxidized form -- dehydroascorbate -- is chemically unstable and readily converted to diketogulonate, which possesses no

Vitamin C activity. Diketogulonate can be broken down into a variety of smaller fragments by many cells. Chief among these metabolites are oxalate and threonate. Some metabolites are excreted, while others appear to remain in the body and perform other functions.

Threonic acid and other aldonic acids profoundly influence Vitamin C uptake and utilization. The studies of Dr. Anthony Verlangieri , Professor of Pharmacology and Toxicology at the University of Mississippi, have laid the groundwork for our better understanding of how these metabolites interact with Vitamin C in the body. His work has shown that C metabolites -- especially threonate -- are directly involved with increased blood levels of Vitamin C. Verlangieri and his colleague, Dr. Michael Fay, have used isolated cells in culture to model how Vitamin C is utilized by various body tissues. They have used cells from laboratory animals as well as humans to study influences on Vitamin C utilization.

In a number of careful experiments, they observed that exposing cells to calcium threonate increased the uptake of ascorbic acid by these cells (Fay & Verlangieri, 1991; Fay, 1992; Fay, Bush & Verlangieri, 1994). Other aldonic acids were shown to have a similar effect (Fay, Bush & Verlangieri, 1994). Verlangieri's group had previously shown that glucose and ascorbate probably share a common transport system for shuttling these molecules through the membrane into cells (Fay, Bush & Verlangieri, 1990). This transport mechanism is thought to involve membrane-bound receptors for ascorbate. These lines of evidence strongly suggest that aldonic acids facilitate cellular uptake of ascorbate by influencing cell membrane receptors.

Numerous studies have shown that orally ingested dehydroascorbate is equivalent to ascorbate as a source of antiscorbutic Vitamin C. Whether ascorbate or dehydroascorbate represents the biologically active form of Vitamin C is an important question that cannot yet be answered. Nearly all of the Vitamin C in the body is present as ascorbate because cells and tissues maintain themselves in a "reduced" state. It is believed that, in order to be transported across cell membranes, all ascorbate must be converted to dehydroascorbate, then converted back to ascorbate within the cell.

## Ester-C® Mineral Ascorbates

The only negative side-effect of regular large doses of Vitamin C seems to be gastrointestinal discomfort and diarrhea. These effects are due in large part to the acidity of ascorbic acid itself. A new technology has been developed to manufacture a Vitamin C product that circumvents many of these problems. At the same time, this new process introduces an important metabolite of Vitamin C so it is present along with ascorbate when

the supplement is taken. The Inter-Cal Corporation of Prescott, AZ utilizes a unique manufacturing process that neutralizes the acidity of ascorbic acid and maximizes the formation of dehydroascorbate and threonate, so they can work synergistically to speed the absorption and utilization of Vitamin C. This technology has received U.S. Patent No. 4,822,816 (Markham, 1989). The ascorbate/C metabolite complex is sold under the tradename Ester-C® ascorbate and is produced in five different mineral forms: calcium, magnesium, zinc, potassium, and sodium.

The composition of the Ester-C® mineral ascorbates has been worked out largely through the efforts of Dr. Seth Rose, Professor of Chemistry at Arizona State University. He applied nuclear magnetic resonance (NMR) techniques to identify various constituents of the Ester-C® products and to quantitate these components for quality control and research/development activities. Dr. Rose's work was instrumental in quantitating the amount of threonate, lyxonate, and xylonate present in the Ester-C® products. High-performance liquid chromatography (HPLC) studies have be used to quantitate the amount of dehydroascorbate present in Ester-C® products. Both NMR and HPLC have confirmed that pure ascorbic acid and pure calcium ascorbate do not contain dehydroascorbate or aldonic acids.

This family of products has been tested in many different situations where high-dose Vitamin C supplementation is used. It is now available for use in a variety of nutritional, topical, dental, and veterinary applications. The way in which Vitamin C is neutralized during manufacturing produces the natural metabolites of Vitamin C that are responsible for so many beneficial pharmacologic actions.

Many studies in humans and animals have been conducted to explore the capabilities of Ester-C® mineral ascorbates and to document their greater effectiveness in the body. These studies are discussed in detail below.

## Animal Studies

In one animal trial, Dr. Verlangieri gavaged two groups of rats with equal amounts of either normal ascorbic acid or Ester-C®. He tested their blood and urine levels of Vitamin C for four hours after administration. The animals given Ester-C® had higher blood levels of Vitamin C, reflecting its improved absorption. Vitamin C was not detected in the urine of the test group until long after it was discovered in the urine of the group given ascorbic acid. This delay in excretion suggests better bodily retention of ascorbate before it is "spilled over" into the urine. Verlangieri's study confirmed that Ester-C® is absorbed more readily and excreted less rapidly than ordinary ascorbic acid (Bush & Verlangieri, 1987). We now know that

the greater absorption and retention of Ester-C® ascorbate observed in their experiments can be attributed to the presence of the threonate metabolite.

Except for some bats, the guinea pig, and monkeys, all lower mammals, including the rat, manufacture their own Vitamin C. However, one strain of rat -- the Japanese ODS rat -- is defective in its ability to manufacture its own ascorbic acid, and it is now widely used by many Vitamin C researchers for laboratory studies of this nutrient. These rats develop scurvy without a utilizable source of Vitamin C in their diets. Drs. Verlangieri, Fay, and Bannon took advantage of this fact to evaluate the bioavailability of Ester-C® calcium ascorbate. To one group of mildly scorbutic ODS rats, they administered Ester-C® calcium ascorbate containing C metabolites. A minimum anti-scorbutic dose of Ester-C® ascorbate was determined after 24 days. When an equivalent amount of ordinary ascorbic acid was administered, they found it not to be anti-scorbutic as assessed by scoring the severity of scorbutic signs, such as morbidity and mortality. This result indicates that Ester-C® ascorbate possesses a higher bioavailability in this sensitive test system (Verlangieri, Fay & Bannon, 1991).

**Human Clinical Studies**

In 1990, Dr. Jonathan Wright and others at the Meridian Valley Clinical Laboratory in Kent, WA reported the results of a human trial of the biologic absorption and retention of Ester-C® ascorbate. The study was designed to compare the absorption, retention, and utilization of Ester-C® and ordinary ascorbic acid. Absorption was measured as Vitamin C levels in the plasma fraction of blood. Retention was estimated from data on urinary ascorbate excretion. And, finally, tissue utilization was assessed by measuring the amount of Vitamin C in the white blood cells, which are known to accumulate and utilize large amounts of Vitamin C for many physiological functions. Wright instructed 12 men to eat a diet low in Vitamin C for one week. He then divided them into three groups of four each. Wright instructed the first group to take a daily high-dose Ester-C® supplement, the second group to take a high-dose ascorbate supplement (providing the same amount of Vitamin C), and a third control group to take a placebo. After a test period during which plasma, white blood cells, and urine levels of Vitamin C were measured four times a day, Wright crossed-over the supplements, giving each of the groups another supplement. In this way, each subject could serve as his own control.

The results of Wright's human study were remarkably similar to those of Verlangieri's first rat study. The plasma ascorbate levels of the Ester-C® group increased 50% over those of the controls and 30% over those of the plain ascorbic acid group. Tissue Vitamin C levels (as measured in white blood cells) of the Ester-C® group measured four times higher than those of

both the ascorbate and control groups. The Ester-C® group also excreted 450% less Vitamin C and 500% less oxalate (the breakdown product linked to kidney stones when excreted in high quantities) in their urine than the ascorbate group (Wright, Suen & Kirk, 1990).

A more focused study of Ester-C®'s cellular uptake was performed recently in San Diego by Dr. Howard Hunt, Professor Emeritus of the University of California San Diego, and Dr. Thomas Rice of the Life Management Group with a group of men enrolled in a corporate fitness program. They wished to see if Ester-C® would significantly increase tissue levels of Vitamin C in infection-fighting and immunological cells, as measured by uptake of ascorbate into the white blood cells. Their research plan was very similar to that of Dr. Wright's earlier study. The subjects began with a 2-week washout period to stabilize their Vitamin C intake at low levels. Three groups of 18 men each then received a one-gram oral dose of ascorbic acid in one of four product forms: ascorbic acid, U.S.P. calcium ascorbate, Ester-C® calcium ascorbate with naturally occurring threonate, or the Ester-C® ascorbate with a standardized 3% calcium threonate level. White blood cells were isolated from blood samples removed at intervals of 0, 1, 2, 4, and 24 hours from the time of administration.

Hunt and Rice discovered that, with time, the ascorbate from all supplements steadily accumulated in white cells, but that Ester-C® ascorbate and the Ester-C® with enhanced threonate reached levels considerably above the final baseline level attained with ordinary ascorbic acid. They concluded that Ester-C® calcium ascorbate/threonate complex provides a superior way to build Vitamin C reserves in the important immuno-competent cells of the blood, even when modest levels (one-gram) of supplementation are used. The standardized threonate group showed further intracellular increases of ascorbate (Hunt & Rice, 1995).

## Veterinary Studies

This improved absorption and retention strongly suggest that the presence of C metabolites confers an amplified physiologic action on Ester-C® compared to ordinary Vitamin C. This conclusion is supported by a growing body of evidence from veterinary practitioners who have begun recommending Ester-C® in place of ordinary ascorbates. Many turned to Ester-C® calcium ascorbate in the first place because it was a gentle way of administering Vitamin C to dogs and horses without gastrointestinal upset. Because Ester-C® was gentler in the stomach of animals, it could be given at the higher doses necessary to achieve desired pharmacologic actions.

Some of the most extraordinary results have been obtained by veterinarians who have searched for innovative and safe ways to treat intractable conditions in dogs and horses. The superior value of Ester-C® ascorbate was recently demonstrated in a Norwegian study of arthritic and dysplastic dogs.

Because dogs are among the majority of mammals which manufacture their own ascorbic acid, veterinarians would not ordinarily think of giving them supplemental Vitamin C. Dogs have been shown to manufacture five-to-six times as much Vitamin C as the Recommended Dietary Allowance for humans, but they still have one of the lowest synthetic capacities in the animal kingdom. Some practitioners speculate that dogs -- particularly older ones -- do not always manufacture optimal levels of Vitamin C and that lower tissue levels of Vitamin C may exacerbate arthritis, chronic joint inflammation, and muscle stiffness.

Dr. Geir Erik Berge, a veterinarian in Oslo, Norway, gave 100 disabled dogs 30 mg/kg of Ester-C® three times daily for six months. To qualify for the study, a dog had to have one of the following chronic conditions involving the joints and connective tissue: severe joint injury, arthrosis, spondylosis, hip dysplasia, older disc-prolapse, muscle atrophy as a result of functional loss, or senile wear-and-tear in support and motion systems.

Dr. Berge measured the success of supplementation in improving the condition of the dogs after one week, six weeks, and six months through clinical evaluations using a scoring system as well as reports from the dog owners. Because of the chronic nature of these joint disorders and their resistance to conventional therapy, Dr. Berge did not include a control group of animals, since their outcomes could have been predicted. Berge's study demonstrated significant results. After one week of treatment with Ester-C®, some 75% of the ailing dogs showed dramatic improvement in their conditions. This percentage improvement remained relatively stable for the rest of the study; by the six-month mark, 78% of the previously suffering dogs had experienced a significant reduction in symptoms. To Berge, the conclusion was inescapable that dogs manufacture suboptimal concentrations of Vitamin C in some tissues under the stress of certain ailments. He recommended therapy with Ester-C® ascorbate to help correct those deficits (Berge, 1990).

Guided by Berge's success with degenerative diseases in dogs, Dr. L. Phillips Brown conducted a similar study with dogs housed at the Best Friends Animal Sanctuary in Kanab, UT. Dr. Brown administed Ester-C® calcium ascorbate, ordinary ascorbic acid, or a placebo twice daily to each group of dogs for three weeks. Response to treatment was graded with the Average Mobility Improvement Score (AMIS), using a four-point scale, with

"0" representing no response and "3" representing a very good response. Treatment was then discontinued for three weeks and the groups were crossed over, so that each group received a different treatment. Treatment and scoring were performed two more times in this fashion. When all improvement scores were added up, Dr. Brown found that dogs receiving the Ester-C® ascorbate showed an AMIS score of 1.5, while dogs receiving plain ascorbic acid showed an AMIS score of 0.5. The average score of the placebo was 0.1 -- indicating that no significant improvement can be expected if no intervention is performed. In addition to showing that Ester-C® calcium ascorbate was about three times more effective than ordinary ascorbic acid for these conditions, Dr. Brown's study also pointed out that improvement of degenerative joint conditions can be expected only when ascorbate supplementation is given (Brown, 1994a, b, c).

Horses also manufacture endogenous ascorbic acid, yet there is growing evidence that the amount produced may not be sufficient to counter the effects of age, husbandry, and athletic demands. Dr. N. Lee Newman realized that Vitamin C supplementation might help with the treatment of degenerative joint disease (DJD) of horses she frequently sees in her large-animal veterinary practice in Middletown, VA. In 1992, she began clinical trials of oral Ester-C® calcium ascorbate for horses diagnosed with a variety of DJD conditions exhibiting moderate to severe lameness. Dr. Newman began to record radiographic evidence and degree of response to treatment using the American Association of Equine Practitioners Lameness Classification Scores. At the conclusion of the 18-month trial period, over 90% of the horses had shown good to excellent response to the Ester-C® calcium ascorbate supplement. More significantly, 80% of the "improved" horses remained sound and usable even after the supplement was discontinued. These results demonstrate the importance of alternative modalities and therapeutics for the treatment of many conditions formerly thought to be hopeless (Newman, 1995).

## Future Developments for Ester-C® Ascorbates

The Inter-Cal Corporation has made a singificant investment in research to understand the composition and biological actions of Ester-C® mineral ascorbates with metabolites. Inter-Cal is assisted by a strong technical staff that provides research support and product quality control. The in-house Research Director heads a team of scientific advisors that guide Inter-Cal's research program into new areas of understanding.

Because of the dismal state of State and Federal funding for basic nutritional and biomedical research, we cannot depend on the research-grant system any longer to support pioneering research in many areas having profound implications for health. In spite of decades of research and

consumer interest, Vitamin C remains a somewhat mysterious nutrient. Many basic questions remain to be answered about how ascorbate functions at the biochemical level and how aldonic acids influence this metabolism at the cellular level. Inter-Cal is actively engaged in sponsoring research studies to elucidate how threonate and other aldonic acids "open the door" into cells through cell membrane receptor interactions. Inter-Cal remains committed to providing corporate funds to support continuing basic laboratory and clinical research into the mechanism of action of Vitamin C metabolites.

## Ester-C® Scientific Literature


Berge, GE. 1990. Polyascorbate (C-Flex), an interesting alternative by problems in the support and movement apparatus in dogs. The Norwegian Veterinary Journal ***102***: 581 [English translation].

Brown, LP. 1994a. Vitamin C (ascorbic acid) -- new forms and new uses in dogs. Proceedings of the 1994 American Holistic Veterinary Medical Association Annual Conference, Orlando, FL. pp. 119-125.

Brown, LP. 1994b. Vitamin C. New forms and new uses in dogs. New Editions HealthWorld, Nov/Dec 1994, pp. 44-45.

Brown, LP. 1994c. Ester-C® for joint discomfort -- a study. Natural Pet ***3 (#6)***: 25-27.

Bush, MJ; Verlangieri, AJ. 1987. An acute study on the relative gastro-intestinal absorption of a novel form of calcium ascorbate. Research Communications in Chemical Pathology and Pharmacology ***57***: 137-140.

Fay, MJ. 1992. Possible biological effects of ascorbic acid metabolites on the cellular uptake of ascorbic acid and other compounds. Ph.D. Dissertation, Department of Pharmacology, University of Mississippi. August, 1992.

Fay, MJ; Bush, MJ; Verlangieri, AJ. 1990. Effects of cytochalasin B on the uptake of ascorbic acid and glucose by 3T3 fibroblasts. Mechanism of impaired ascorbate transport in diabetes. Life Sciences ***46***: 619-624.

Fay, MJ; Bush, MJ; Verlangieri, AJ. 1994. Effect of aldonic acids on the uptake of ascorbic acid by 3T3 mouse fibroblasts and human T lymphoma cells. General Pharmacology ***25***: 1465-1469.

Fay, MJ; Verlangieri, AJ. 1991. Stimulatory action of calcium L-threonate on ascorbic acid uptake by a human T-lymphoma cell line. Life Sciences ***49***: 1377-1381.

Hunt, H; Rice, T. 1995. Comparative efficacy of Inter-Cal "Ester-C®" calcium ascorbate. Results of a study prepared for Inter-Cal Corporation, March 1995 by The Life Management Group, Inc., 3262 Holiday Court, Suite 204, La Jolla, CA 92037.

Markham, Richard G. 1989. Compositions and methods for administering Vitamin C. United States Patent Number 4,822,816 issued 18 April 1989.

Newman, NL. 1995. Equine degenerative joint disease. A nutritional approach with Ester-C®. Natural Pet ***4 (#2)***: 56-57.

Verlangieri, AJ; Fay, MJ; Bannon, AW. 1991. Comparison of the anti-scorbutic activity of L-ascorbic acid and Ester C in the non- ascorbate synthesizing Osteogenic Disorder Shionogi (ODS) rat. Life Sciences ***48***: 2275-2281.

Wright, JV; Suen, RM; Kirk, FR. 1990. Comparative studies of "Ester C" versus L-ascorbic acid. International Clinical Nutrition Review ***10***: 267-271.

## Inter-Cal Scientific Advisory Board

*Chairman*

**Jack Hegenauer, Ph.D.**
Associate Research Professor
Department of Biology
University of California San Diego

Research Director,
Inter-Cal Corporation

*Advisory Board Members*

**Anthony J. Verlangieri, Ph.D.**
Professor of Toxicology and Pharmacology
Department of Pharmacology
School of Pharmacy
University of Mississippi

Director,
Atherosclerosis Research Laboratories
University of Mississippi

**Steven J. Silverstein, D.M.D., M.P.H.**
Professor,
Department of Dental Public Health and Hygiene
School of Dentistry
University of California San Francisco

**L. Phillips Brown, D.V.M.**
President,
The Natural Connection
South Yarmouth, MA

**Robert M. Hackman, Ph.D.**
Associate Professor of Nutrition
Department of Anthropology
University of Oregon

**Howard F. Hunt, Ph.D.**
Professor Emeritus,
Department of Physical Education

University of California San Diego

President,
Life Management Groups, Inc.
La Jolla, CA

**Thomas B. Rice, Ph.D.**
Professor Emeritus,
Department of Health and Physical Education
San Diego Mesa College

Consultant,
Life Management Group, Inc.
La Jolla, CA

**Hugh D. Riordan, M.D.**
President & Director,
Center for the Improvement of Human Functioning Intl.

Director,
RECNAC Cancer Research Center
Wichita, KS

**Jeffrey S. Bland, Ph.D.**
Chief Executive Officer,
HealthComm, Inc.
Gig Harbor, WA

**Richard Passwater, Ph.D.**
Director of Research,
Solgar Nutritional Research Center
Berlin, MD

**Geir Erik Berge, D.V.M.**
Olso, Norway

**Michael E. Rosenbaum, M.D.**
Marin County, CA

# Better Hunting With Vitamin C

*By Larry Mueller, Hunting Dogs Editor*

*If muscle soreness cuts your dog's performance short, try vitamin C. It works with many human beings, and I've found that dogs benefit, too. Why quit hunting halfway through the day because your dog tires too quickly?*

All right men," Johnny Stevens shouted, "hunt 'em up!" The "men" were 16 Beagles, male and female, but that didn't matter to Johnny. They had all had their vitamin C that morning, just like me. I had asked my Tennessee buddies, "Would you like to hunt in the name of science to test vitamin C for dogs?" and Roger Sear's response was: "We'll hunt in the name of *anything*!" So there we were, about to learn if vitamin C can prevent muscle soreness in dogs the same way it does in many human beings.

If you read "Hillybilly Hounds" in the December 1985 issue, you already know the hunters. They turn something fairly ordinary—rabbit hunting—into adventure by running a large pack of hounds that function as a unit and fill the hills with music.

I've known for years that vitamin C prevents muscle soreness in humans, and the pack was large enough for an objective test on dogs.

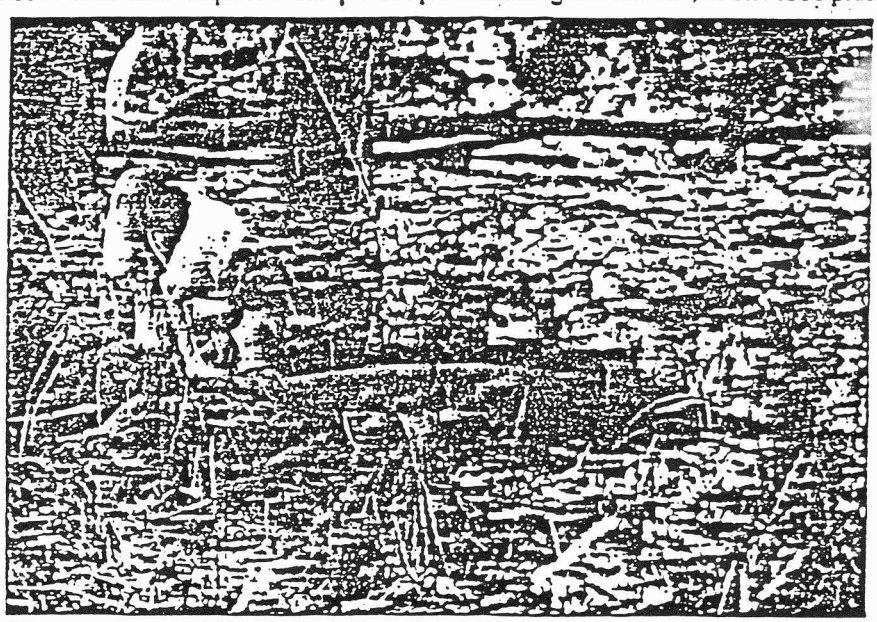
Vitamin C has never worked as well for me after I was sore as when I took it before, during, and after the hunt. The Beagles on the "Hillbilly Hounds" hunt were already tired and sore from two days on cottontails when we dosed them with vitamin C. Then came the hardest day—hunting swamp rabbits in heavy, brier-infested cover on an island in the Tennessee River. We decided to try vitamin C anyway, but as Johnny helped me dose the beagles, I thought he had the worried look of a man who suspected he was administering dope or poison.

With the heat of midday, one by one, the Beagles began to quit. They were so tired, hot, and sore that they curled up and went to sleep wherever they stopped. I could tell Johnny was blaming the vitamin C. Remembering the handshake that nearly broke my trigger finger, it didn't seem wise to argue. And I was sure I'd never get another chance to try vitamin C on that pack.

### Other Experience

I also told my bear-hunting friend Earl Bostick about vitamin C. It's difficult for Earl to keep his Plott Hounds conditioned in Illinois so that they can hunt bears in Ontario, so Earl was anxious to try it. I called him after his annual hunt. Did it work? You bet! The hounds held up better than they had in all the previous years Earl had trekked north for bears. But Earl was interested in a good bear hunt, not science. It had all sounded logical to him, so he had dosed every hound before, during, and after each day's hunting. Since there were no untreated hounds, there was no scientific comparison, only Earl's impressions. While I've known Earl long enough and well enough to trust his judgment, I knew that others would not regard mere observation as fact in the absence of a real test.

In the meantime, I heard about a study funded by the Alpo Pet Center. Dr. David Kronfeld, Clark Professor of Nutrition at the University of Pennsylvania School of Veterinary Medicine did the testing with sled-dog racer Harris Dunlap. Dunlap divided 36 dogs into groups of four each on the basis of sex, age, and similar running ability. Four different diets were fed: 1. Complete and balanced canned food only; 2. The food plus 500 milligrams of vitamin C; 3. The food plus 30 milligrams of zinc; 4. The food plus



*Without vitamin C, Beagle is ready to quit after only a few swamp-rabbit chases. Thick cover, mire, and canecutters exhausted the dog.*

Blood samples were taken at the start of training, just before racing season, just after the North American championships, and four weeks after the last race. Dunlap evaluated each dog's stress tolerance and performance. Those dogs with vitamin C in their diet performed best, and performance correlated with the concentration of vitamin C in the blood. Zinc had little or no effect.

I can't personally correlate my own vitamin C experience with the many facets of stress, but I know that large amounts of vitamin C increase my endurance by eliminating most of the muscle soreness due to extreme exercise. I hoped it would do the same thing for hunting dogs, and Dr. Kronfeld's study was very encouraging.

The Test

I checked back with the Tennessee beaglers about another try. To my surprise, even Johnny was willing. He had given it a lot of thought and had tried it on himself while cutting firewood. He found it worked marvelously, and he was no longer worried about giving it to dogs. Vitamin C is water soluble, and any excess is excreted in the urine, so it's safe.

We couldn't do Dr. Kronfeld's blood tests, so we had to rely on performance evaluation of the hounds by men who knew the hounds well. I listed the dogs, paired them by ages, and asked about characteristics of each one that might influence the outcome.

Eight received vitamin C; eight did not. I calculated the dosage by multiplying the amount I must take to get good results by the fraction formed by dividing my weight into an average Beagle's weight. Our Beagles got 500 milligrams before the hunt, 500 milligrams whenever we could catch them in the middle of the hunt, and 500 milligrams after hunting. That's three times the amount given to the much-larger sled dogs in the ALPO study.

It was noon before we could dose the eight dogs a second time on the first day, later than I preferred. They were already showing fatigue and disinterest caused by poor tailing conditions in the midday heat. We stopped for lunch and let them rest. None of the hounds ranged out the search when we resumed the hunt. But one Beagle jumped a swamp rabbit, and fatigue was forgotten. All 16 hounds left in full cry. The last chase of the day was one of the longest. The hounds frequently went out of hearing. Three of the day's chases had lasted 45 to 60 minutes.

The hounds were all tired that night, but we wouldn't know until morning whether muscle soreness had begun to take its toll. At load-up time in the morning, none of Tommy Runnels's hounds seemed stiff. Johnny's dogs all came out, but Spot, a dosed dog, was most eager. His age-mate, Joe, was more reluctant, but he was a harder searcher and had probably expended more energy. Roger's Rip, a dosed dog, was anxiously waiting at the gate. His age-mate in the test, Pee-Wee, had to be called out.

The difference between them widened greatly by the second morning. Pee-Wee had to be pulled out of the doghouse.

The third morning dealt the experiment a severe blow. Both Johnny and Tommy so their hounds didn't run. Although Roger's Daisy was running, she was no longer a valid part of the comparison because her age-mate, Hank, had been terrified by the boat ride to the island where we hunted swamp rabbits and refused to hunt on the first day. Roger's Jim was dosed, and he started and trailed the first rabbit of the third day long before undosed dogs joined in, but he no longer had an age-mate for comparison. Jay Greene teamed up with us on the third day, so we still had a large pack, but we found ourselves reduced to only Rip and Pee-Wee as valid age-mates in the test. Rip was at the gate again. Pee-Wee had to be pulled out.

Roger joined me after I shot a four-pound swamper that doubled back on its track once too often. "The amazing thing," Roger said, "is that Rip and Pee-Wee reversed their positions in the pack. Pee-Wee is taller and better built for speed, so he's always out front and barking nearly every breath. Rip is small and usually runs third or fourth in the pack. Rip is right there in his usual place, but now Pee-Wee is running behind him and barking only once every 10 or 15 feet."

On the fourth morning, Roger and I were the only hunters, and we were down to two dogs. Rip was still jumping through cover, tail wagging. His age-mate, Pee-Wee, looked as if he was walking on eggs. His tail was still, and he wouldn't hunt.

We shot one rabbit over Rip and quit, disappointed that all 16 hounds hadn't been in the entire test, but thoroughly convinced that vitamin C does indeed improve hunting-dog performance and endurance by reducing muscle soreness. For those who would like to try it on their dogs, the amount we used was 500 milligrams for each 20 or 25 pounds of body weight. Give this dose three times daily to keep the vitamin in the bloodstream. (One big dose would be lost in the urine long before the end of the day.) Regardless of what breed of bird dog or hound you hunt with, if you try this, please write me about the results so we can report again in a future column: Larry Mueller, Box 143, St. Jacob, IL 62281.

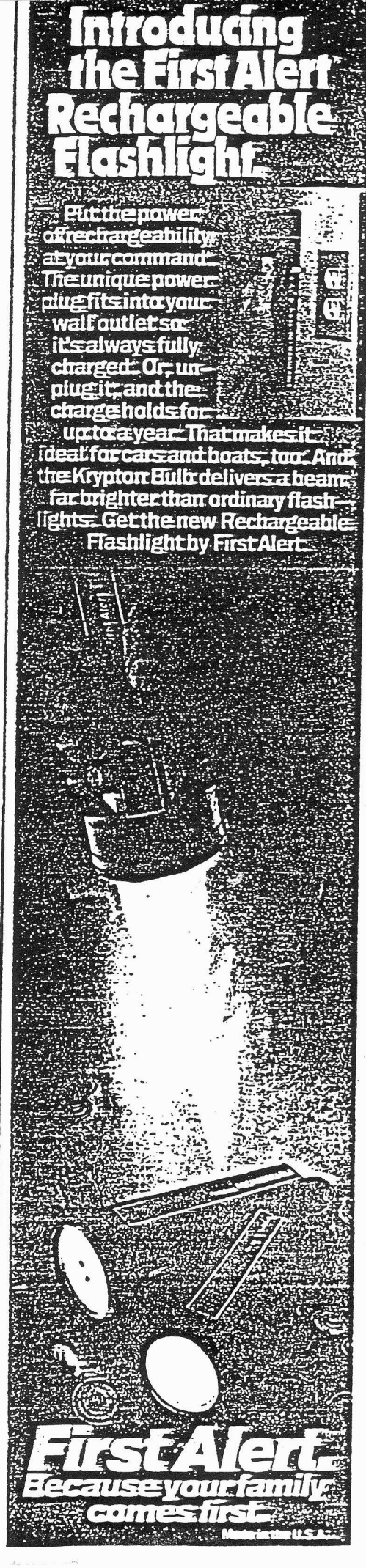
## Book Of Classic Hunting Tales

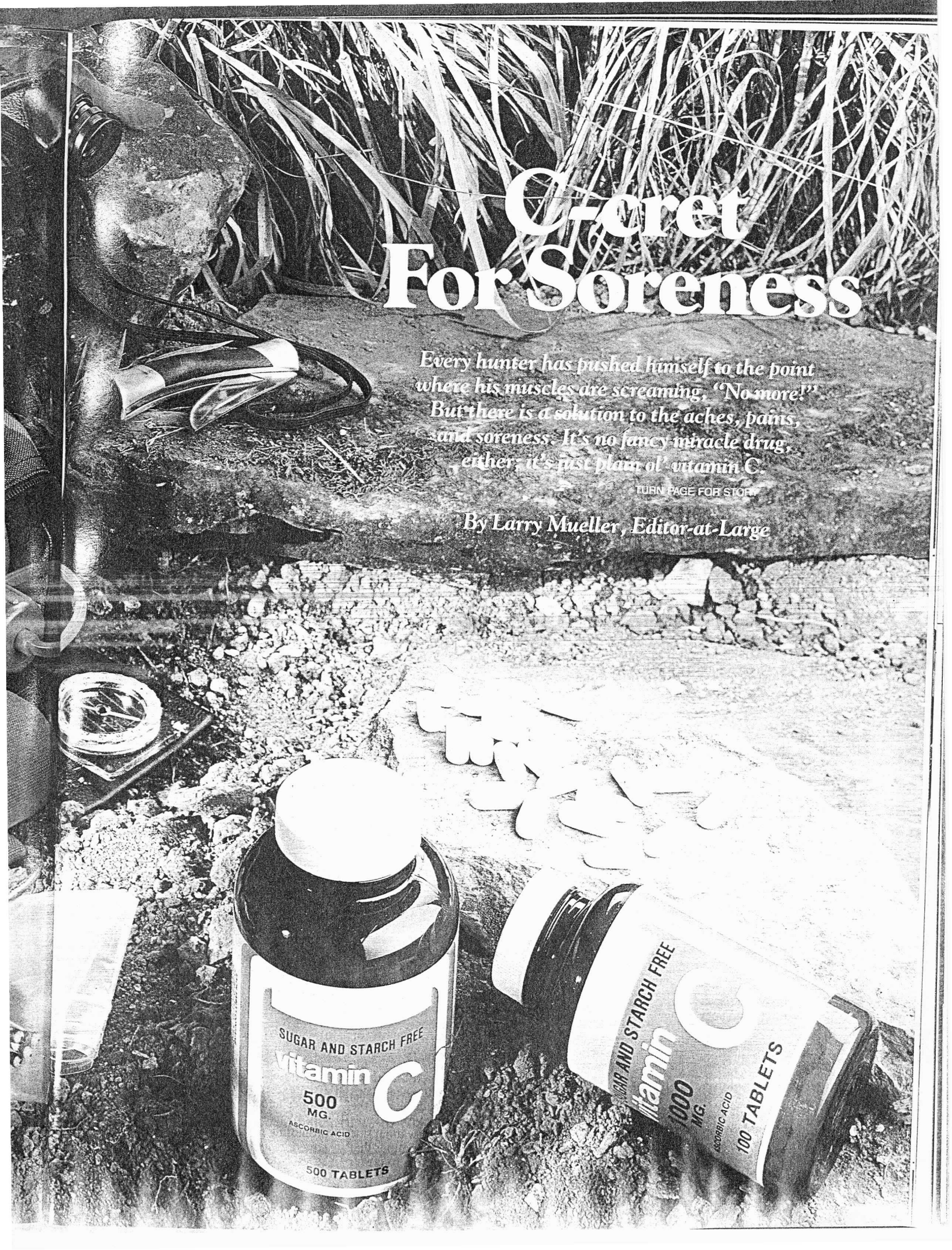
*The Greatest Hunting Stories Ever Told* a collection of classic hunting tales that were selected and edited by OUTDOOR LIFE Executive Editor Vin T. Sparano, is now available.

The new 265-page hardcover book offers a total of 28 hunting stories from such well-known authors as Robert Ruark, Teddy Roosevelt, Nash Buckingham, Pat McManus, Ben East and many others. Their tales will pull you swiftly from page to page, whether you're duck shooting in Tennessee or on safari with Jack O'Connor as he hunts leopards, rhinos and lions.

Many of the tales in *The Greatest Hunting Stories Ever Told* were out of print until now, making this anthology a valuable volume for collectors of hunting literature.

Autographed copies of *The Greatest Hunting Stories Ever Told* are available for $19.95 postpaid from Vin T. Sparano, 17 Henning Dr., Fairfield, NJ 07006.



Introducing the First Alert Rechargeable Flashlight.
Put the power of rechargeability at your command. The unique power plug fits into your wall outlet so it's always fully charged. Or, unplug it and the charge holds for up to a year. That makes it ideal for cars and boats, too. And the Krypton Bulb delivers a beam far brighter than ordinary flashlights. Get the new Rechargeable Flashlight by First Alert.
First Alert.
Because your family comes first.




# Secret For Soreness

*Every hunter has pushed himself to the point where his muscles are screaming, "No more!" But there is a solution to the aches, pains, and soreness. It's no fancy miracle drug, either; it's just plain ol' vitamin C.*

TURN PAGE FOR STORY

*By Larry Mueller, Editor-at-Large*

The straps of muscles behind my knees were screaming, "Buddy, you quit right now, or spend the next three weeks off your feet entirely." And it was only noon of the first day! There were 2½ days of chukar hunting left. I had looked forward to this for months. But how would I keep up with companions who were 15 to 25 years my junior; who were carrying just shotguns while I had to pack two cameras, lenses, and film; who were used to 3,000-foot slopes so steep that we often walked sidehill on our ankles; and who were used to slide rocks that slipped from under the feet like banana peels?

Probably every hunter has pushed himself too far and wished he hadn't—especially during those muscle-flabby early days of the season. I've done it so often that I've finally started paying attention to the warning signs. But I was on a hunt 2,000 miles from home, and I desperately wanted to continue.

Suddenly, it dawned on me. The solution to my problem might be back at the vehicle in my case of extra camera gear. It was a little late to remember, and I should have started this before the hunt. But just maybe I had found the cure for my pains.

Back at the vehicle for lunch, I rummaged through my gear. Hooray! The bottle was there. You're supposed to begin taking these things *before* extreme exercise, not wait until you're sore, but I washed down six tablets anyway. And I pocketed six more to take three or four hours later. If they didn't work, someone would have to carry me back because I intended to hunt.

There was no immediate improvement. But by midafternoon, the soreness was gradually subsiding. Thereafter, I gobbled six tablets (500 milligrams each, making a total of three grams) before breakfast and another six about every four hours, adding up to 12 grams each day. By the early afternoon of the third day, I was the only one *without* sore leg muscles. In fact, my young buddies were hurting badly enough to halt the hunt a few hours early.

What kind of magic did I have in that bottle? Pain killer? Miracle medicine? Illegal drugs?

None of the above. Just vitamin C. A weight lifter taught me this about 20 years ago. Pumping iron five hours every other day makes muscles *hurt*. My friend learned, however, that if he took vitamin C before and after exercise, he could work out without the extreme soreness.

He was using 500 mg before and after, so I tried it. The supreme test came when I managed a week's vacation early in hunting season. We hunted ducks at daybreak, quail and pheasant for the balance of the morning, rabbits in the afternoon, and raccoons at night. Tired? Every night, my air mattress went flat, and I never knew it until morning. Yet, I experienced no real muscle soreness, just the pleasant tiredness that a man in good shape (although I wasn't) feels after a long hunt.

Over the years, however, I've had to increase the amount of C for it to work effectively. Maybe it's because I'm older. Maybe it's because I get into rougher terrain these days. I suspect, though, that each person has to find his own dose at his own time in life, perhaps influenced by his own current lifestyle. I've heard, for example, that a smoker needs an extra daily 1,000 mg of C just to help repair the damage caused by his habit. So does a woman on birth-control pills, or anyone exposed to toxins.

I also suspect that I'm using C more freely and more effectively now than I did 20 years ago because I have since learned more about it. At first, I approached the idea hesitantly. I mean, who wants to look like a pill popper? But I finally got it through my head that the shape of the vitamin was fooling my thinking processes. If someone pressed beef into a tablet, would that make it medicine? Of course not!

Obviously, I checked its safety before consuming large amounts. Vitamin C is water soluble, so there's no danger of buildup in the body. Whatever the body doesn't need at the moment is cast off in the urine. There are, however, a few minor things you should know. I doubt that you'll go into the hospital for diagnostic tests and hunt on the same day, but the accuracy of such tests may be thrown off by large amounts of C. Also, some people experience mild diarrhea when their bodies receive more C than their systems need at the moment. You may urinate a bit more than usual, and a rare few may experience a little stomach upset. I could discover no side effects of consequence for normally healthy humans taking large amounts of C—certainly nothing like the serious side effects that sometimes accompany many of the medical drugs that people take without question.

*By the early afternoon of the third day, I was the only one without sore leg muscles.*

How does vitamin C prevent muscle soreness? Surprisingly, I found that chances are strong that your doctor can't tell you. Most members of the medical profession have not been very well educated in nutrition. I put the question to two doctors teaching medicine at two different universities. They had no idea. I thought that surely, research people at the companies making vitamin C tablets would be knowledgeable. The response everywhere was, "There's nothing about that in the literature." A private practitioner hit me with the placebo theory: Give a group chalk or sugar pills, and one-third of them will get relief from pain.

"It's not something to ignore," he told me, "but neither does it work often enough to be reliable. And I suspect that your vitamin C experiences are placebo effect."

I thought about that. But if power of suggestion worked that well on me, why did I get to be 19 before pills ever cured one of my headaches? Two aspirins never have. Two APCs given to me by a Navy doctor were the first. Later, I learned that three five-grain aspirins will cure my headaches and leave me feeling dragged out for the rest of the day. Three (not two) Tylenols will cure most of my headaches without side effects. Get the point? With variations that specific, how could I be reacting to power of suggestion?

Finally, by accident, I found James Braly, M.D., in Encino, California. Dr. Braly (pronounced Braw-ly) is both a nutritionist and an allergy specialist. In light of the general ignorance I encountered regarding vitamin C, I found Dr. Braly's grasp of the subject astounding.

"The soreness we feel after extreme exertion," said Dr. Braly, "is caused by microscopic tears in the muscle. The body has to repair these tears cell by cell, and soreness continues until the job is finished. To begin with, these cells are held together by a substance called collagen, which acts something like mortar between bricks. The body is dependent upon vitamin C to produce this collagen.

"Vitamin C is delivered to the repair site by the bloodstream, where C is concentrated in neutrophils, or white blood cells. If the neutrophil doesn't have enough C to do the job, it will even rob other parts of the body. If general levels of C remain too low, soreness continues."

Obviously, if the bloodstream is provided adequate C before the hunt, rebuilding of the cells begins as quickly as microscopic muscle tears occur, and soreness is minimized or even eliminated.

Dr. Braly went on to say that the body's response to inflammation (whether due to infection or exertion) is also dependent upon the adrenal glands. C is heavily concentrated in the adrenals, which cannot function at optimal levels without adequate amounts of the vitamin.

In addition, vitamin C is among the nutrients important in the formation of the hormonelike chemicals called prostaglandins, which are produced from the membranes of every cell in the body. There are about 40 different prostaglandins in several categories, or series, that must be balanced for good health and a feeling of well being. Series 1 and 3 prostaglandins are health-promoting; those in series 2 are actually illness-promoting. The health-promoting prostaglandins are vitamin C-dependent. If they can't get adequate amounts, the body is more sensitive to pain, does not heal as quickly, and is therefore more prone to soreness.

Shortly after talking to Dr. Braly, I gave vitamin C one more chance to work on my adrenals, and neutrophils, and cells, and collagen, and health-promoting prostaglandins. I was pounding the whole mess to a pulp against a saddle while hunting mountain lions in southwestern Texas with merciless, ranch-born Bill McKinney, who rides like he's a piece of the animal that just disconnects and walks around at times.

Bill's wife, Bonnie, was more sympathetic, if not more encouraging, when that first evening I found it almost impossible to sit at her supper table. Herself a lifetime horsewoman, Bonnie said, "I hate to tell you this, but by morning, you'll be [illegible] more sore. But go ahead and ride tomorrow, anyway. The soreness will work out."

Well, this was the first time that my "microscopic tears" got that far ahead of the large doses of C taken before, during, and after exertion, but I went back to the camper and took three more grams anyway. By morning, to my surprise, my health-promoting prostaglandins (or should I say *posteriorglandins?*) were back in charge. No aches. No pains. Once again, C was the secret for soreness.

# Dysplasia's End

*I believe that if you take some simple steps to prevent it, you never have to own a pup with hip dysplasia.*

*By Larry Mueller, Hunting Dogs Editor*

Roughly one in four hunting-dog pups become victims of hip dysplasia. Some of these dogs suffer a lifetime of crippling pain. Others are destroyed. But it is now a virtual certainty that if you take some preventative measures, your pup will not get hip dysplasia. Is that a strong statement? Read on, and judge for yourself.

**Scurvy And Hip Dysplasia**

San Jose, California, veterinarian, Dr. Wendell O. Belfield, was visiting a friend. On the table was a reprinted copy of the 1753 book *A Treastise On Scurvy*, by Capt. James Lind, a surgeon in the British Royal Navy. While waiting for his friend, Dr. Belfield began reading. The symptoms described were: bleeding gums, loose teeth, foul breath, nose bleeds, swollen eyelids, brittle bones . . . . And then came an autopsy report made in 1699 at St. Louis Hospital, Paris: "The ligaments of the joints were corroded and loose. Instead of finding in the cavities of the joints the usual sweet oil mucilage, there was only a greenish liquor; which by its caustic quality had corroded the ligaments."

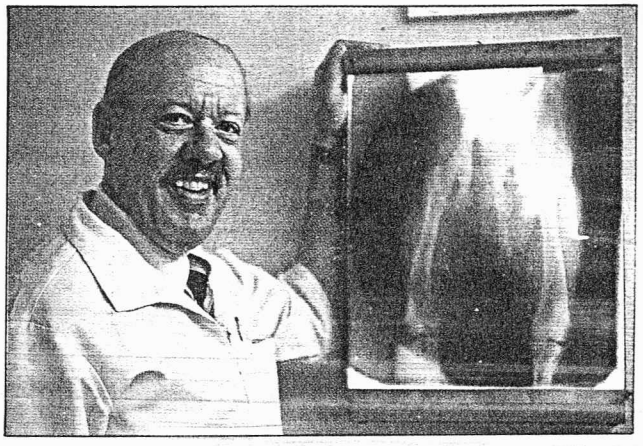
Recognition flashed across Dr. Belfield's mind. This 300-year-old autopsy of a dead human being described hip dysplasia in dogs. Could chronic hip dysplasia be a form of scurvy? Simply a lack of vitamin C?

No, it's accepted that a dog's body makes its own vitamin C. Everybody knows that. Canine hip dysplasia is hereditary. Everybody knows that, too.

But why does everybody know that dogs don't need extra vitamin C? Because back in the 1930s, some dogs in testing laboratories suffered no ill effects when denied all vitamin C in their diets.

We can't help but observe, however, that very little happens in the controlled environment of an experimental laboratory that would cause dogs to need more C than its body can manufacture. In the real world, a dogsled racer brought a bitch to Dr. Belfield after other vets in the San Francisco area had failed to help her. She had a swollen tongue, bleeding gums, and often fell and dragged herself about with great difficulty.

*Dr. Wendell O. Belfield, who pioneered dysplasia prevention, examines patient for weak joints.*



*Dr. Belfield points out hip area where dysplasia develops. X-ray is really best for diagnosis.*

Dr. Belfield recognized the trouble as scurvy, although not the true clinical scurvy that brings death to humans. The dog was making enough C to keep the scurvy on a chronic subclinical level. After a month on ascorbic acid (another name for vitamin C—simply meaning *not* having scurvy) the bitch could run as long and hard as any dog on the client's team.

Why didn't other members of the racing team have scurvy? They had been eating the same food. This happened because of another factor usually ignored by those who believe that dogs never need more C than their bodies make. In one group of 104 dogs tested, vitamin C levels varied from .02 milligrams per cubic centimeter of blood to .84 milligrams —a 42-fold variability! Obviously, one dog's body can be starving for C while another dog makes enough to get by.

This variability also leads us to the heredity factor. Breeding programs to eliminate CHD have worked to a certain degree. For instance, the Wirehaired Pointing Griffon breeders eliminated half of the problem in just 10 years through selective breeding. But no breeding program has come close to being 100 percent effective. If we assume that Dr. Belfield is right in believing that hip dysplasia is connected to a vitamin C deficiency, it all begins to make sense. The variable ability to make ascorbic acid is certainly inheritable. While selecting parents for lack of hip dysplasia in the heredity, we may have unknowingly selected for the real factor that inhibits CHD—good vitamin C production.

**Nature And Nurture**

It seems clear, however, that all CHD is not entirely genetic. Selective breeding has been too inconsistently successful to

→

believe otherwise. Dr. Belfield says that he constantly sees sound parents. A Swedish doctor who X-rayed army dogs concluded that dysplastic dogs produce only 10 percent more dysplastic pups than normal dogs. But again, if we accept Dr. Belfield's vitamin C theory, it all makes sense.

Vitamin C does 300 different jobs in the bodies of animals, including humans. One of the most important is collagen production. If you think of cells as bricks, collagen would be the mortar. Without enough collagen, you can't build muscle tissue. You can't build bone, either, because collagen forms the honeycomb holding the minerals in place in bones. Furthermore, it's possible to have not quite enough vitamin C to manufacture quality collagen. Weak collagen builds weak muscles and bones.

A second function of ascorbic acid is to cope with the effects of stress. It does this by nourishing the adrenal glands and by helping the body produce its own cortisone, which combats histamines produced by dying cells. A rat can multiply its vitamin C output tenfold when stressed. Humans can't make any at all, much less increase it, so we must eat all that our bodies need or supplement our diets. Apparently, our ancestors ate huge amounts of fruit containing vitamin C, so unlike most animals, our livers didn't develop the ability to turn glucose into ascorbic acid. Dogs are poor producers. A goat can make five times the vitamin C produced by a dog of equal size. A rat, small as it is, makes nearly four times the vitamin C produced by a full-grown dog.

I hate the term "conventional wisdom." Too often, it means believing what everybody else believes, no matter how foolish, for fear of being ridiculed. But in this case, the definition is accurate. Conventional wisdom has it that dogs need no more vitamin C than their bodies make. Dr. Belfield asks why, if that is the case, do dogs or wild canines, when left to their own devices, act like they need more? When possible, canines do eat fruits, berries and vegetation containing vitamin C.

In my experience, there are two times when dogs eat large amounts of grass and other available vegetation. One is when they've run long and hard, and there's no water to be found. At such times, they chew and swallow vegetation for the moisture. The other time is when they've been kenneled too long. Even some hard-running bird dogs and hounds often stop and eat green vegetation before taking off to hunt. We've always wondered why. We even thought up several explanations, but they didn't ring true. Could this simply be caused by a need for additional vitamin C?

Keep in mind that there is no extra vitamin C in most commercial dog foods. That's not a criticism, either. Ascorbic acid oxidizes rapidly when the lid is off the container. It probably would oxidize rapidly as a minor ingredient in a large bag of food. Also, the high heat during the extruding process would probably destroy most of the vitamin C added to the food. One manufacturer that does add ascorbic acid makes no claims for its benefits, nor is the amount specified or guaranteed to be in the bag.

To sum up so far, our dog is a poor producer of vitamin C, there is no extra C in his usual food, and like us, he needs far more in proportion to size than we do to achieve normal growth. While our growth is stretched out over two decades, most of theirs takes place during the first year. The bigger the breed or strain, the more rapid the growth, and the greater the demand for ascorbic acid, the greater the incidence of hip dysplasia.

Dr. Belfield suggests that we should also consider the stress assault on a domesticated pup. It's weaned, separated from its mother and littermates, involuntarily carried to a new and unfamiliar location, stuck with needles, mildly poisoned to eliminate worms, and possibly operated on to remove dewclaws or part of the tail. All of this happens while the animal is already stressed by teething and phenomenal growth. The growth factor induces a very high demand for ascorbic acid through additional stress and the massive need for collagen.

*The autopsy of a human scurvy victim described canine hip dysplasia.*

The wild canine pup, by contrast, stays with its mother, keeps its tail and dewclaws, suffers no early separation, is not hurt by hypodermic needles, and has not been bred overlarge by foolish humans. Diseases and worms are its stresses. But the wild pup does get extra vitamin C from the livers of animals the mother kills, some green vegetation, and sometimes from fruits and berries. The domestic pup gets none because we have decided that he doesn't need any.

Our pup—in a condition perhaps bordering on subclinical scurvy—runs, plays and jumps with muscles and bones weak from lack of quality collagen. Muscle growth may not be keeping pace with bone growth. The pectineus muscles become taut. Suddenly, during one great leap or unusual jolt, the weak muscles fail, and the balls of the leg bones are pulled away from the hip sockets. The lubricating synovial fluid leaks out, and the balls grate on the sockets. In hours, maybe less, the dog has hip dysplasia. It will not recover.

**The Solution**

Dr. Belfield thought that the solution was obvious. Prevent hip dysplasia by supplying enough vitamin C. He tried it with several litters of German Shepherd pups, a breed with serious CHD problems. The parents either had hip dysplasia themselves or they had already produced dysplastic pups.

The first bitch had very bad hips, and according to Seeing Eye Dog standards, should have been neutered to prevent damaging pregnancy. Dr. Belfield gave her 2,000 milligrams (2 grams) of C daily as soon as she was pregnant. Eight pups were born, and they were given 50 to 100 milligrams of liquid C from birth until weaned. From that point to four months, 550 milligrams of powdered C were added to their food. This was increased to 1,000 milligrams, then 2,000, until the pups were 18 to 24 months old. None of the pups were dysplastic.

Another bitch had been bred twice to different studs that were certified free of CHD. Each time, half of her pups were dysplastic. She was bred again and vitamin C was administered under Dr. Belfield's guidance. She produced 11 pups with perfect hips. In all, eight litters were handled in this manner over a five-year period. When X-rayed at two years of age, all pups were free of CHD.

The testing ended in 1976, and as far as Dr. Belfield was concerned, the CHD threat was defeated. He published a paper in a professional journal.

Was it truly over? Hardly. Dr. Belfield had attacked the popular genetic theory that everyone "knew." Instead of simply trying the treatment and finding out for themselves, most of the professionals ridiculed Dr. Belfield for not conducting a better experiment with double blinds (some dogs not given C). He was even called a crook and a charlatan.

"That really hurt," Dr. Belfield told me. "I'm just a little one-man practitioner who looked for better ways when conventional means weren't working. Proof is up to the universities. I really want to help these animals, not wait around for further proof while the dogs suffer."

Those are my sentiments exactly. Dr. Belfield has since upped the dosage of C somewhat and has combined it with other vitamins and minerals. He also mixed some with Ester-C after learning about our beagle experiment. (See my August 1986 column in OUTDOOR LIFE.) I'm currently testing both mixtures on hard-running foxhounds, which you'll hear about soon. In the meantime, should you want to try the mixture Dr. Belfield uses, or read the book he did with Martin Zucker, send 25¢ for more information to Dr. Wendell Belfield, 3091 Monterey Rd., San Jose, CA 95111.

Whatever you do, if you're raising a litter this spring, provide the needed vitamin C. If you're buying a pup, put it on C the moment you take it home. There's no sense risking hip dysplasia just because absolute proof hasn't yet been established or seriously attempted.

*Autographed copies of Larry Mueller's book* Speed Train Your Own Retriever *are available from: Retriever, Box 143, St. Jacob, IL 62281 for $17.95 postpaid or $19.95 shipped first-class mail.*

# Miracle Cure for Hip Dysplasia?

*A form of vitamin C is gaining a reputation among veterinarians for putting lame dogs back on their feet again.*

By Larry Mueller
HUNTING DOGS EDITOR

An acquaintance of mine, B.J. Richardson, was calling from Texas, doubt and hope in his voice. "My English pointer isn't a year old, and he's already lame in the rear end, especially the left hip," Richardson said. "The X-rays show hip dysplasia. The veterinarian says there are two choices: Operate to alleviate the pain or put the dog down. I can't afford one and won't do the other. Is it true that vitamin C might help?"

I had to say that I'd never heard of vitamin C curing canine hip dysplasia, but I was aware that veterinarian Wendell Belfield of San Jose, Calif., did prevent CHD—or at least its symptoms—in eight litters of German shepherds, a breed that is prone to the crippling abnormal development of a dog's hip joints. In those instances, all of the dog parents had CHD or had previously whelped pups that became dysplastic. Belfield gave the bitches vitamin C throughout pregnancy and lactation. The pups received vitamin C from weaning until they were two years old. None of pups developed CHD during that entire period.

Though Belfield's work wasn't scientific in the strict sense, it certainly indicated that CHD could be prevented. Still, I couldn't see how the joint could be remodeled once it had grown improperly, at least not without surgery. However, vitamin therapy seemed to be Richardson's only hope, so I told him what I knew.

Many readers had written and told me that their arthritic dogs normally were laid up after a few hours in the field, but when given vitamin C, they could hunt several days in a row. None had said they did it with dogs that had CHD, but maybe...

I also recalled reading about the efforts of Dr. Bob Cathcart, a medical doctor in California who championed the use of vitamin C in curing a wide variety of joint ailments and illnesses. Much of his work centered around using the vitamin in large quantities, increasing the doses until the body reaches "bowel tolerance." Though Cathcart's work was with human patients, many veterinarians adopted his method, saying that vitamin C should be given in increasing doses until the dog's stools loosen, at which point the dose should be backed off a half gram or a gram at a time until the stools became firm again. At that point, the dog's body receives the maximum vitamin C that it can utilize.

I also understood that a superior form of the vitamin is Ester-C, which can be purchased in health-food stores. The vitamin in Ester-C is molecularly locked to calcium, so it doesn't cause the acidity problems normally associated with ascorbic acid (the common form of vitamin C), which can upset a dog's stomach. Ester-C also has natural C metabolites that get it into the cells faster and more effectively (common ascorbic acid is slower getting out of the blood serum, so it passes through the kidneys, where much of it is rapidly lost in the urine).

## Pinto's Rebound

A month or two later, I heard that Pinto, Richardson's dog, had begun improving less than a week after receiving maximum doses of Ester-C. Pinto, the grandson of Miller's Chief—an 11-time champion in horseback-style bird-dog trials—was now running like the wind. I was as surprised as I was delighted.

Two years later, I was in Texas and dropped in to see Pinto. Richardson had kept him on a maintenance dose of Ester-C. The dog was moving with a fluid grace and power in the hips. Twice, for a step or two, I saw a bunny hop, suggesting that not everything was 100 percent correct. But both times, Pinto immediately shifted back to a normal gait.

I still couldn't understand how Ester-C could remodel a defective joint, but I was hopeful. Nobody I knew whose debilitated dog had improved clinically on Ester-C had ever taken X-rays of the joints, so I asked Richardson to

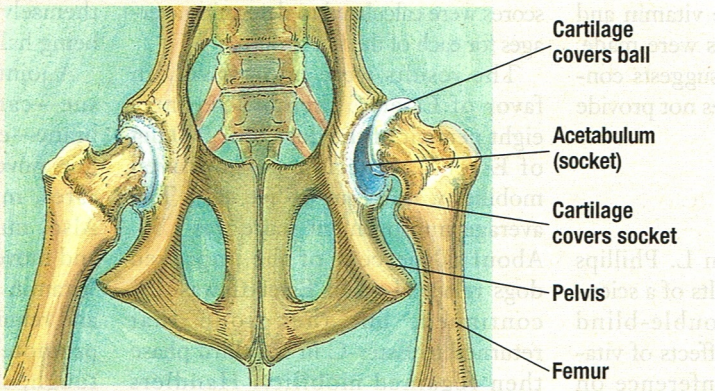
**PINTO, an English pointer, was diagnosed two years ago with severe hip dysplasia. His owner had two options: surgery or euthanasia. Today, the dog shows no outward signs of discomfort.**

have X-rays taken.

He did and mailed me the original X-ray taken two years before and a new one. I showed both to Diana K. Stuckey, a board certified radiologist in St. Louis, who looked at the original and pointed out the hip dysplasia with the left hip most severe. The second? "Arthritis that customarily follows hip dysplasia," she said.

I explained Pinto's quick and lasting response to Ester-C. "How could this dog go from lame to moving freely, and apparently without pain, in a few days—and stay that way—without something improving in the joints?"

"We occasionally see this," Stuckey said. "A dog is arthritic yet moves as if it feels no pain. We don't know why. Great 'heart' maybe, or high pain tolerance."




**A NORMAL hip joint (left) is compared with a dysplastic one. Cartilage helps pad the joint.**

## Mystery Unfolds

I'm sure that veterinarians do see this. But the answer bore little logical connection to my question. Pinto's improvement was not because of great heart or high pain tolerance. He had been hurting and he had been limping badly. If his response to such pain improved in just a few days, something caused that change.

Dr. Chuck Noonan of Weston, Conn., also compared the X-rays. He noticed slight improvement in the severity of the dysplasia but said the hip joint had clearly succumbed to degenerative arthritis from the dysplastic hip joint banging around in and out of the socket.

"Eighty-three percent of dysplastic dogs either show an improvement in their hip dysplasia or they learn to deal with the problem as they grow older," Noonan said. "The second X-ray shows that the dysplasia is slightly less severe, but because of the arthritis, the joint is worse overall than in the earlier X-ray. It is possible that the vitamin C was helping to sort of lubricate the joint so the dog felt less pain."

In my investigations, I had found that Pinto's results from Ester-C weren't unique. Soon after Richardson first called, I received a letter from Steve Dudley of Arizona. His young black Lab, who showed great promise at hunting Gambel's quail, went lame with CHD. Dudley's vet suggested that Dudley replace the hip—or expect to put the dog down by age four.

Dudley tried Ester-C instead and the dog promptly improved. Kept on Ester-C, the dog lived until age 13 without showing signs of soreness, lameness, or unwillingness to hunt, Dudley wrote.

## Flood of Proof

My investigation also led to Charles Docktor, an Arizona veterinarian who was the first to test Ester-C for its effectiveness in healing joint problems. In 1983, he used Ester-C on a large number of arthritic dogs, finding that 75 percent improved in various degrees in a short period of time.

Independently, a continent away, Dr. Geir Erik Berge, a veterinarian in Oslo, Norway, performed a similar study that was reported in the August-September 1990 issue of *The Norwegian Veterinary Journal.* Berge selected 100 dogs with a variety of joint ailments. His testing revealed that 75 percent of the dogs rapidly improved on Ester-C, some only slightly, some almost totally. Dr. Berge added that large amounts of vitamin C metabolites, substances essential to a body's metabolic processes, are required in rebuilding diseased joint tissue.

Corroborating data were also reported by Dr. N. Lee Newman, who conducted 18 months of clinical tests using Ester-C to combat degenerative joint disease in performance horses. She reported a remarkable 90 percent success rate, ranging from good to excellent. Furthermore, 80 percent of the improved horses remained sound after Ester-C was discontinued. Newman credited supplemental Ester-C with maintaining the integrity of collagen and connective tissue and with mobilizing white cells in the immune system, while deactivating free radicals that damage cell membranes.

But other respected voices were making contradictory statements. The Cornell University College of Veterinary Medicine *Animal Health* newsletter in May 1995 denied that vitamin C was of any value for either preventing or treating skeletal diseases in dogs. "There have been absolutely no confirmed reports that vitamin C is helpful in any such instances," the newsletter stated. It went on to theorize that supplemental vitamin C has no value because dog's produce adequate amounts of the vitamin in their livers.

But that reasoning is questionable. Vitamin C production varies from dog to dog, individual bodily needs vary, and circumstances—health and environment—vary enormously. "Adequate" in human medicine only means enough C to prevent scurvy. What is adequate for a strict carnivore like a dog? And in any case, "adequate" should not be assumed to be a synonym for "optimum."

This is where a vitamin C standoff occurs, and getting people to change their scientific opinion is often like asking them to change their religion. In Cornell's favor, the evidence that has existed supporting the use of vitamin C on dysplastic dogs is heavily anecdotal. Even the various veterinarians' research that has been cited was actually efficacy

tests—that is, all of the dogs tested were given similar doses of the vitamin and no controlled comparisons were made. Efficacy testing strongly suggests conclusive evidence, but it does not provide true scientific proof.

## The Acid Test

But in 1994, veterinarian L. Phillips Brown presented the results of a scientifically acceptable "double-blind crossover" study on the effects of vitamin C to a national conference on holistic veterinary medicine. Brown, the owner of the largest veterinary hospital on Cape Cod for 22 years, tested vitamin C on 50 dogs with serious joint problems. The dogs were among a population of more than 500 canines at a large animal sanctuary in Utah.

It should be noted here that representatives of Inter-Cal, makers of Ester-C, specifically asked Brown to study the vitamin because they felt it could have a major role in the treatment of joint abnormalities. Dave Stenmoe, one of the representatives of the manufacturer says, "We told [Brown] not to take our word for anything," he said. "Just to keep an open mind and conduct a scientific comparison of Ester-C, ordinary vitamin C, and a placebo. He finally agreed to do it."

Brown, along with the Utah sanctuary's resident veterinarian, hand-picked the dogs with the worst cases of joint disease and placed them in five groups (see chart).

Every dog had a handler who knew the animal and evaluated behavioral change weekly on the basis of 0 for no change, 1 for slight improvement, 2 for moderate to good improvement, and 3 for very good mobility.

After four weeks of testing, the supplements were withdrawn for three weeks. Then, each dog was crossed over to a different group and received another supplement for another four weeks. After yet another three-week layoff, 60 percent of the dogs were switched to a third supplement. The remaining 40 percent went back to whatever they were given during the first four weeks. At the end, mobility scores were calculated to determine averages for each of the five groups.

The results were impressively in favor of Ester-C therapy. Seventy-eight percent of the dogs on 2,000 mg of Ester-C experienced improved mobility within four or five days. The average improvement score was 1.52. About 60 percent of the improved dogs relapsed when Ester-C was discontinued, but the group that returned to Ester-C in the third phase then regained mobility. Handlers reported no negative side effects.

On the low (850 mg) dose of Ester-C, only 52 percent of the dogs improved, with an average score of 0.45. Obviously, size of dose was important. Of dogs receiving 2,000 mg of Ester-C with extra minerals, 62 percent improved by an average score of 0.87. Why Ester-C without extra minerals had better results remains unknown.

**Brown's Double-Blind Crossover Study Results**

| Compounds (Doses) | Dogs w/ Improvement | Average Mobility Rating (out of 4) |
|---|---|---|
| Ester-C (2,000 mg) | 78% | (1.52) |
| Ester-C (850 mg) | 52% | (0.45) |
| Ester-C plus minerals (2,000 mg) | 62% | (0.87) |
| Ascorbic acid (2,000 mg) | 44% | (0.67) |
| Placebo | 4% | (0.04) |

Ordinary vitamin C improved 44 percent of the dogs, with a score of 0.67. As expected, no noticeable change occurred among dogs on the placebo.

Not even the most dyed-in-the-wool skeptic can ignore the results of such a double-blind crossover study. But the success of vitamin C in treating CHD can still be questioned, or even denied, because X-rays show that the joints remain loose or arthritis remains. Even Brown confirms that X-rays taken for his study reveal defective skeletal structures even after the Ester-C treatment.

## Soft Tissue Factor

But those who see improvement with Ester-C are looking primarily at an animal's behavior—they see an improved ability to function. How can both proponents and skeptics consider themselves correct? Perhaps by each being half right.

A joint is not bone alone. Soft tissue—cartilage and synovial membrane—exists between bones to permit movement. If such tissue deteriorates, movement becomes painful. Also, muscles atrophy from disuse, and movement becomes more painful. Vitamin C is essential in the making and rebuilding of soft tissue because it promotes the growth of collagen, a tough, stringy "mortar" that holds cells together. At the same time, the soft tissue also holds water, which maintains compression resistance to cushion the joint—this is the "lubrication" described by Noonan in his assessment of Pinto's X-rays.

In healthy cartilage, normal cell loss is balanced by the rebuilding of cells. Under diseased or inflammatory conditions, cell loss is excessive. In the case of a dog's hip joint this can mean that adequate cushioning no longer exists. The high demand for vitamin C may begin exceeding the amount made in the dog's liver, so deterioration continues. Or supplemented vitamin C may turn the process around.

Field experience, although still anecdotal, suggests that dogs on Ester-C lead full lives without terrible pain and debilitation. Ester-C may prove to be a wondrous holistic cure, but OUTDOOR LIFE cautions that it's too early to state definitively that vitamin C can cure or rectify canine hip dysplasia. Some doctors contend that the treatment is merely a Band-Aid on a far more serious problem.

We should add one point. Hip dysplasia is at least partially inheritable. And it is not a simple, single-gene defect. There is concern that dysplastic dogs returned to mobility may also be returned to reproduction, which would further spread the malady. It is fair to say that there appears to be a great deal of hope for the benefits of C, but before administering the vitamin to your dog, consult your veterinarian. And until more is known, don't breed that dog.

# New C Insight

*There weren't many sore spots in the results of our test on the effects of Vitamin C on hard-working hunting dogs.*

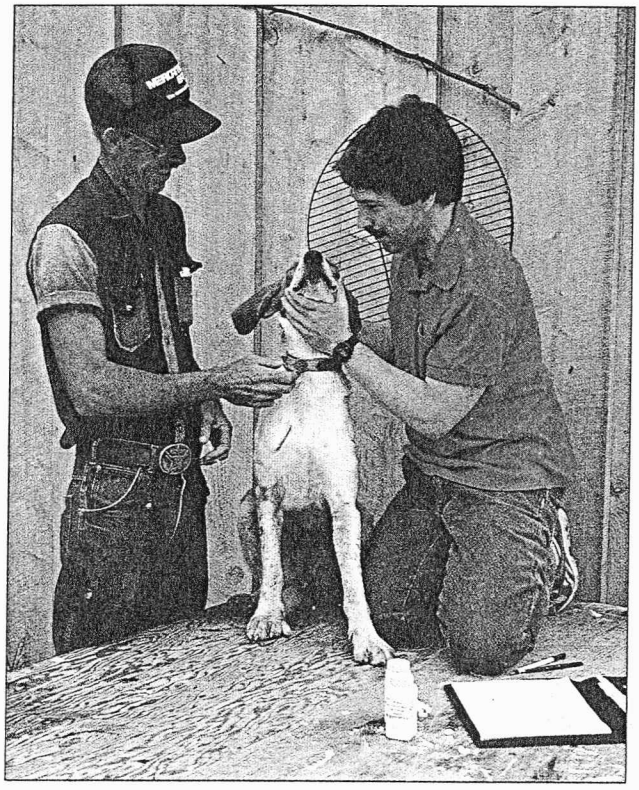
*By Larry Mueller, Hunting Dogs Editor*

"She's not going to come to you," Laura Murry yelled to her husband, Terry. "Her tail is down!"

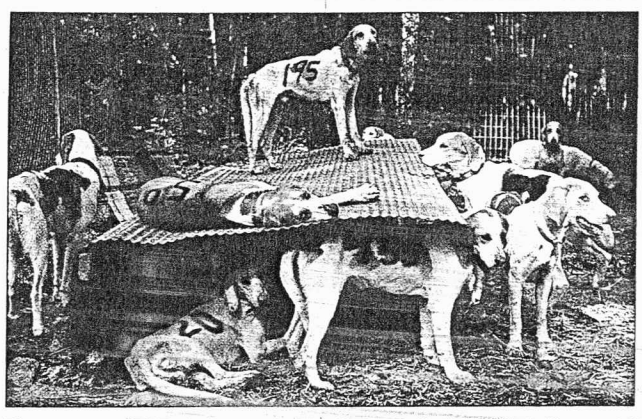
My ears shot straight up. This girl is aware, I thought! I jockeyed for a better position to see through the bushes. Sure enough, Molly's tail was straight out at the base, but the last one-third to one-half dipped downward. This is a canine expression of indecision—when a dog is apprehensive or in doubt.

In this case, Molly was going up the steep side of a ravine. Terry was on the rim trying to call her. The dog was having her doubts about obeying, wondering whether she'd be able to slip past Terry and rejoin the other foxhounds. Molly tried maneuvering back and forth about 30 feet below the rim, but Terry always managed to stay directly above her, coaxing her to come.

Finally, Laura called, "OK, Terry, you can catch her now. I saw a circle in her tail."



*Alan Coates (left) helps ALPO's Mark Finke administer Vitamin C to one of the participants.*



*Some of these "trail tough" hounds didn't seem to need extra C to get them through the test.*

Over the rim came Molly, tail curved up and wagging as she trotted straight to her master.

I was delighted. Keen perception was extremely important in the Vitamin C test that we were conducting. In the past we had compared Beagles given C to Beagles that had gone without C. Later we had learned about a sort of super C (Ester-C, now more accurately called polyascorbate), which according to lab tests, enters the stomach pH-neutral (causing no acidic upset), gets into the bloodstream twice as fast, stays in the body at least twice as long, and is four times as bio-available while in the white blood cells. I had found both forms of C—when given in doses of 70 milligrams per pound of weight per day—effective in preventing the serious muscle soreness that usually results from extreme exercise. But I had been looking for a way to make an actual comparison of ordinary C and polyascorbate in the field. Fortune had smiled on me when I stumbled onto the Murrys at a dog event.

I've known Terry and his dad, Bill, for many years, but Laura was new in the family. So was, I soon learned, an interest in foxhounds. Terry had discovered that foxhounds are the speed and endurance athletes of the hunting dogs world. Laura was raised in a fox-hunting family and has had a lifelong passion for the chase. In fact, her son by a previous marriage is named Chase. When I met up with them, the Murrys owned 54 foxhounds.

Here was the perfect chance for comparison. Terry and Laura were excited about the idea, as well, so we made a pre-→

liminary test using vitamin/mineral supplements mixed by Dr. Wendell Belfield. After I had written about his use of Vitamin C in preventing hip dysplasia, Belfield had mixed ordinary C in one batch of supplements, polyascorbate in another, and asked me to compare them on dogs.

The Murrys were running 30 hounds in a 375-acre fox pen (fenced enclosure), and there was about enough of each mixture to treat 10 dogs. The Murrys reported no change in performance from the untreated dogs, somewhat of an improvement in dogs getting the "white powder" (they didn't know which mixture contained what) and the most spectacular results with the dogs on "brown powder." The brown contained the polyascorbate.

When out of the Belfield mixtures, we continued the comparison with the powdered forms of polyascorbate and ordinary C sprinkled on the food. Sometimes the Murrys ran their hounds as much as three days in field trials. They won the Illinois state championship. Again, the polyascorbate was "brown," and almost immediately the Murrys became so convinced of that powder's superiority that they wouldn't use the "white" at all during field trials.

What impressed the Murrys most was their hounds' new and unusual recovery time. Foxhounds are given to excesses. If running was whiskey, they'd be the drunks of the canine world, as these dogs often drop at the point of exhaustion and sleep it off.

"When we used to come home from a run," Terry said, "we'd have to pull them out of the truck and almost drag them to their houses. They'd possibly take three days to recover. Now, on Ester-C, they jump out of the truck looking for something to eat."

Some of the hounds also improved their endurance by as much as two hours.

Then disaster struck. Terry was severely injured by a falling boulder in a coal mine. Before he could recover fully, the mine closed. The Murrys had to sell more than half of their hounds. And before the tests could resume, the objective had changed. I had been back East and had met Mark Finke, director of nutrition at ALPO. Mark had been interested but skeptical. He really didn't believe that Vitamin C is useful to dogs, much less that one form is better than another, but he'd agreed to fly out to observe if we'd run ordinary ascorbic acid against a placebo. I was excited. Here was a chance to convince a scientist (who has the means to continue the comparisons in the lab) that Vitamin C is important to hard-working dogs.

We arranged a three-day lease of the Divide Fox Pen—1,300 acres enclosed by six miles of fence near Doniphan, Missouri, about 28 miles from the Arkansas border. A pharmaceutical contact of Mark's would supply both Vitamin C and placebo tablets. Nobody would know which was which until after the test. Terry would arrange to have 30 hounds—15 well-matched pairs—at Doniphan on June 20.

Things began to unravel on June 19. The Murrys didn't own 15 matched pairs, and the friend who was to supply the balance canceled at the last minute. Alan Coates, manager of the pen, quickly rounded up more than enough local hounds to fill the quota. Some pairs, however, couldn't be properly matched. Owners of six hounds couldn't be present to evaluate the performances of their own dogs. Three hounds got loose before they could be given either the C or the placebo. We had 35 hounds on the ground, but only 12 pairs that might be evaluated.

Heat was a further complication. We knew that the hounds wouldn't be able to run long into the day because of the temperature,

*Here was a chance to convince a scientist that Vitamin C is important to hard-working dogs.*

so we turned them loose at 2 a.m. the first morning. I would have preferred an earlier start, but some of the hunters were afraid that we'd lose the hounds too soon in the test due to exhaustion. That proved to be incorrect. Daylight came at 5 a.m., and hounds were quitting as early as 6 a.m., obviously far from exhaustion.

Although some of the hounds were capable of running for 12 hours, they were again quitting as early as 6 a.m. on the second morning. That's when it dawned on us that when these hounds are run at night, they're accustomed to being picked up soon after daybreak. They were staying with the chase until the fox went to ground, but if it was daylight by that time, not many hounds were seriously looking for another animal.

A 1 a.m. start on the third morning was little help. The majority quit by 5:30 a.m. Interestingly, Red didn't stroll in until 10:36 a.m. Gracie was out until 11:10 a.m.! Both hounds belonged to Alan. Red was one of the three that had gotten loose before the test began. He received no C at all. Yet Red ran for three days and was the second to last dog in! Keep this fact in mind.

Other strange things happened. The Murry hounds were accustomed to running on dirt and mud, not rocks. Their pads wore badly. Fergy's were the worst, despite Laura's frequent treatments. On the third morning, Fergy went out of the gate with the other hounds, but she whimpered with each step. Fifty feet from the pen she fell. Terry stepped forward to catch her, and just as he did the pack struck hot scent and raced away screaming like sirens—with Fergy right behind as if nothing at all was wrong with her pads. And she ran for three hours! Furthermore, she was on placebos, not C. Remember this too.

After watching behavior that seemed to contradict all expectations, the owners were supposed to study their hounds' performances and decide which dog in each pair had gotten the Vitamin C. Don Klepzig, owner of the pen, was correct on a pair of 14-month-old youngsters and wrong on a pair of 2 1/2-year-olds. Roy Pigg was correct on both pairs of 9 1/2-month-old pups, but was in doubt about Jill—a three-year-old who had been teamed with Alan's Gracie—who he thought had run normally.

Terry knows his hounds, but watching Laura work reminds me of a story from the 1800s in which a New Mexico rancher was permitted to accompany a group of Apaches on a deer hunt. The group was following deer tracks at a rapid rate, but Montague Stevens saw nothing unless the ground was bare and the prints obvious. Each time he'd motion a complaint that he couldn't see anything, the annoyed Indians would stop and point to perhaps one bent blade of grass. Laura is the kind of person who sees the bent blade or single hair out of place. On the first day she easily picked three hounds that were on C. On the third day, just after casting, Laura had a more difficult time deciding, but made a note of three more that she thought were on C. She was correct again.

Mark Finke and I positively were not seeing the bent blades. Even Terry argued with Laura about some hounds. Earlier tests had shown me strong black-and-white evidence that Vitamin C works, but this was not that cut and dried. Why?

I puzzled over my notes for weeks afterward. Finally, several things came together in my mind. The inconsistencies began to make sense, and I believe we discovered something new about Vitamin C.

I've noticed that when I go from writing all day to hunting all day, my unconditioned body requires a full 12 grams of C (divided in four separate doses) to avoid serious muscle soreness. After the second or third day, however, I tend to forget some of the doses. Evidently at that point I've come back into condition and don't require as much.

Susan Butcher, four-time winner of the Iditarod sled dog race, told me in a recent interview that the rules call for a mandatory 24-hour rest period to be taken anywhere along the 1,158-mile route. She takes hers after the second or third day.

"Even though you've trained to run your team 100 miles and more a day," Susan said, "you're not conditioned to do that *every* day. You're not yet what we call 'trail tough.' But after those two or three difficult days and a rest, the balance of the race seems easier."

Though the physical demands and degrees of conditioning are quite different for my activities as compared to Susan's, the same two or three days are necessary to become trail tough. After that, we can both go on with what we do for many days. But every time we lose conditioning through a layoff, we go through the same experience. Roughly the same thing happens to dogs. If we can keep adequate nutrition in them, however, and if Vitamin C can keep them from breaking down from muscle and joint soreness, they, too, can get past those first

severe days and go on almost indefinitely.

This certainly explains why most of the Missouri hounds were impossible to judge, plus why Red and Gracie were last to quit. In or out of the pen, these hounds run several nights a week. They stay trail tough and need no extra C to get them through.

The Murrys can't run their Illinois dogs that frequently, so their hounds don't stay trail tough. Then why, you ask, did footsore Fergy—not on C—run another three hours? Why did even the perceptive Laura have some difficulty judging which of her dogs had been given C? And why were the Missouri hunters correct on all three pairs of hounds younger than 14 months, though wrong about half the time with the older dogs?

*If running was whiskey, foxhounds would be the drunks of the canine world.*

Young hounds are not in condition, or truly trail tough, until they're fully muscled. They, more than mature dogs, need Vitamin C to help maintain muscle and joint tissue to avoid soreness. Because the benefits are more obvious in young dogs, perhaps Laura and Terry saw the differences more clearly back when their hounds were younger. The differences were definitely clearer in the Missouri youngsters.

Fergy is three years old and fully muscled, thus better physically and mentally equipped to go ahead and run despite adversity. The attention span is far better in mature dogs than in pups. Also, as Mark Finke points out, no one knows what degree of stimulus will motivate individual dogs. We saw a lot of stimulation in the fox pen—far more than on the outside where game is more scarce.

In summation, I believe that young dogs undergoing extreme exercise have a greater need for additional C than mature dogs; unconditioned mature dogs benefit greatly from extra C, as it helps them get over the two or three-day barrier to trail toughness without significant pain; and healthy dogs kept conditioned to hunt more than three days in a row may not need supplemental C at all.

Ever the objective scientist, Mark says that I may be right, but he remains skeptical. The perceptive Laura says that results would have been discernible with Ester-C. Another field test, minus the mistakes of this one, is planned for polyascorbate during cold weather. Meanwhile, if you want more information about Ester-C, contact Inter-Cal Corp., 421 Miller Valley Rd., Prescott, AZ 86301 (602-445-8063).

*Autographed copies of Larry Mueller's books* Speed Train Your Own Retriever *($17.50 postpaid, $19.95 first-class) and* Speed Train Your Own Bird Dog *($19.95 postpaid, $21.95 first-class) are available from the author at Box 143, St. Jacob, IL 62281.*

## Book Of Classic Hunting And Fishing Tales

*Tales Of Woods And Waters* is a brand-new collection of hunting and fishing stories that have become classics. This huge 380-page hard-cover volume, edited by OUTDOOR LIFE Executive Editor Vin T. Sparano, features 37 stories by such legendary writers as Gene Hill, Burton Spiller, Jack O'Connor, Pat McManus, Robert Ruark, Havilah Babcock and many others. These memorable stories span more than 80 years of hunting and fishing literature. This newest and biggest collection by Sparano is custom-bound with a slip case.

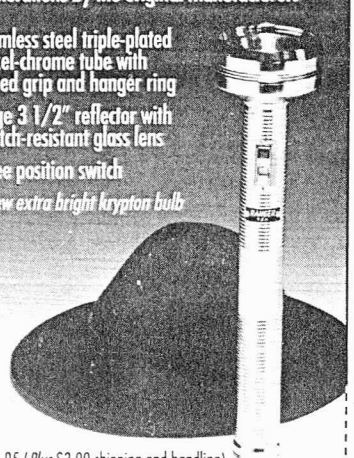
Autographed copies of *Tales Of Woods And Waters* can be ordered for $34.95, plus $2 postage, from Vin T. Sparano, 17 Henning Drive, Fairfield, NJ 07006.


**The Famous 5D-Cell Ranger Searchlight**
**—An American Classic**

Made in the USA in the same way it has been for generations by the original manufacturer.

- Seamless steel triple-plated nickel-chrome tube with ribbed grip and hanger ring
- Large 3 1/2" reflector with scratch-resistant glass lens
- Three position switch

With new extra bright krypton bulb



1 @ $14.95 (*Plus* $3.00 shipping and handling)
2 @ $26.95 (*Plus* $5.50 shipping and handling)
3 @ $37.95 (*Plus* $7.50 shipping and handling)

Add appropriate state sales tax. Please send _____ Rangers.

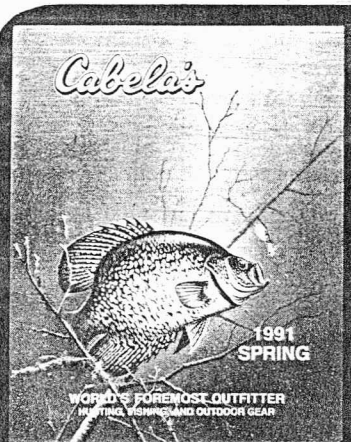
Name _____

Address _____

City _____ State ____ Zip _____

Send check or money order to:

**American Classics**
P.O. Box 25, Sewickley, PA 15143




**Cabela's®**
**WORLD'S FOREMOST OUTFITTER™**
**HUNTING, FISHING, OUTDOOR GEAR**

**FREE**
**CATALOG SUBSCRIPTION**

Rods . . . Reels . . . Lures . . . Boating
Camping . . . Outdoor Clothing . . . Hunting
Footwear . . . . Gifts . . . . and Much More!

100% SATISFACTION GUARANTEED

**FREE CATALOGS WILL ARRIVE THROUGHOUT THE YEAR!**

Send a free subscription to your friend.

NAME _____

ADDRESS _____

CITY _____

STATE _____ ZIP _____

Yes, Please Send My Free Subscription

NAME _____

ADDRESS _____

CITY _____

STATE _____ ZIP _____

MAIL TODAY TO RESERVE YOUR FALL ISSUE!

Cabela's®

801-012th
Sidney
Nebraska 69160

# Super-C For Hunters And Dogs

*By Larry Mueller, Hunting Dogs Editor*

*Vitamin C does a lot to prevent muscle soreness so that you don't have to quit hunting when you or your pooch gets stiff. Now, however, there's a new form of vitamin C that does even more. It even helps with pain caused by hip dysplasia and arthritis.*

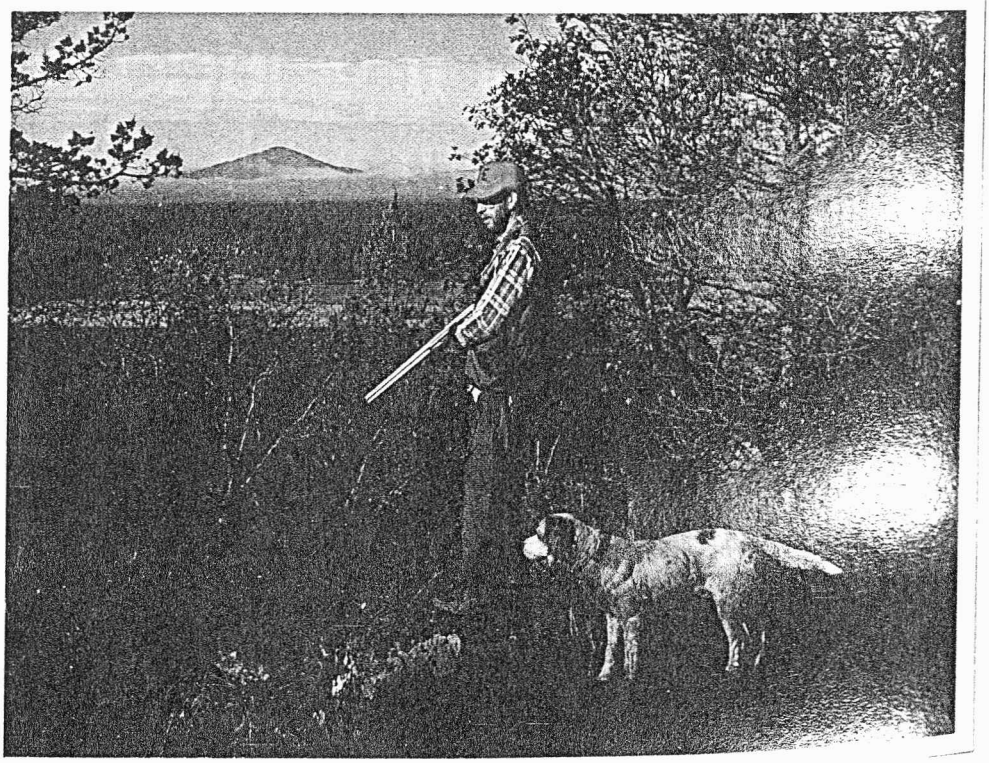
If you read my column, "Better Hunting With Vitamin C," in the August 1986 issue of this magazine, all I can say, friend, is, "You ain't heard nothing yet." Now, we have super-C, and it works to prevent muscle soreness in hunters and dogs in ways I hadn't dreamed about. Let me bring you up to date with what has been done with ordinary vitamin C (ascorbic acid) bought right off the drugstore shelf.

Frank Duncan, a Kansas resident, read my article about extending dog endurance by using C to minimize muscle soreness, and he responded to my request for reports from those who might try it. Frank is a firefighter and works 24 hours on and 24 off; he has either three or five days off in a row every two weeks. He hunts almost every day he's off and was using aspirin in a futile effort to combat the stiffness this heavy hunting caused in the hind legs of his three-year-old Brittany. This past season, Frank gave his bitch just one 500-mg tablet of ordinary vitamin C each morning. She was stiff only *one* night during an entire season that began with doves and early teal and didn't end until Duncan hung up his gun in March.

Jim Stametz of Pennsylvania reported that his six-year-old Beagle became stiff and sore any time he hunted her two days in a row. He now gives her 250 mg of C morning, noon and evening and hunts her four or five days in a row. She becomes normally tired, Jim told me, but exhibits no signs of stiffness.

Herman Feller's German Shorthair came up lame after a three-day Wisconsin grouse hunt the year before last and was in trouble throughout the pheasant season that followed. This past season, depending upon the number of hours in the field, Feller gave his 11-year-old bitch one to three 1,000-mg tablets of C. She hunted the three-day grouse kickoff plus nearly every day →

*Tom Scott and 11-year-old Setter, Speck, [illegible] country that's a challenge even for a young dog. Thanks to vitamin C, Speck hunted hard.*



of the pheasant season and never once came up lame.

Texan Bert Lloyd came a little closer to using what I call super-C. He chose buffered C (calcium ascorbate) to make it easier on his Pointer's stomach. The deteriorated hip joints of Bert's seven-year-old bitch had been replaced four years previous. She was fine for two years. Then, she started favoring her right foreleg and would become very stiff after a day's hunt. It always took four or five days for her to recover enough to hunt again. Cortisone and aspirin were of little help. Lloyd gave her buffered C during the past season, and she hunted for 40 days without any stiffness.

Gerald Elders, a Brittany owner in Prescott, Arizona, had no story about his dog. Instead, in a very polite way, Gerald suggested that where vitamin C was concerned, I was still driving a Model T. Modern C, he told me, is chelated and esterified. At the time, I wasn't even sure what those terms meant. I've since learned that "esterified" means linking the molecules of ascorbic acid. "Chelating" means tightly bonding those molecules to calcium. I've also learned that ordinary calcium ascorbate (buffered C) is not bonded. It separates in the stomach, where the ascorbic acid forms gas bubbles. The calcium fights the discomfort of this gas, so it's better than ordinary unbuffered C, but not nearly as easy on the stomach as chelated, esterified calcium ascorbate—or, for short, ester-C.

I was a bit skeptical, of course. Everyone claims to have a better product. But nothing impedes human progress like a closed mind. I resolved to pay attention and reserve judgment. After all, Gerald Elders wasn't asking me to accept anything on faith. He was saying, "I'll send you some to try. You'll see the difference."

Fair enough. I gave a quantity to Donald Spencer, a doctor of veterinary medicine, to try in his practice, and I took some along on a California hunt. Sure enough, I promptly dumb-donkied into a big need for it. Tom Scott's English Setter, Speck, is now 11. The dog flew as air freight with us on Eastern Airlines to Reno, Nevada. Then, after some brief exercise, the old dog stayed in the airline crate during the two-hour drive to meet John Boitano in Westwood, California. Speck slept the night in the same small space. Right out of that cramped condition, we turned loose the Indiana flatland dog to hunt the mountains on a day that began with cold rain. To complete Speck's troubles, Tom and I were both so excited about going afield with a man who actually hunts mountain quail, not just an opportunist who shoots one or two while hunting something else, that both of us forgot to give vitamin C to the dogs.

That night, Speck was as limber as the Statue of Liberty. Too late, I thought, I dug out the ester-C. We had no hope that Speck would be able to hunt again during that trip. I only hoped to shorten the duration of the old dog's soreness.

The next morning, to our surprise, Speck became highly agitated when he realized that he was being left behind. "OK, OK," Tom finally said, "we'll take you along. You can stay in the vehicle." We gave Speck another 1,000 mg of ester-C.

Out on the mountain, Speck wanted no part of staying in the vehicle. Again, Tom relented, knowing that he'd probably carry the old dog back. By noon, however, Speck was still hunting and appeared far less stiff than he'd been the night before. After another 1,000 mg, Speck hunted until the evening, improving steadily despite the day's exertion.

Boitano's Vizsla was tiring a bit after two days, so it was a good excuse to switch to his young English Setter. Speck, now on 1,000 mg of ester-C three times a day, kept pace with the far younger dog.

*Super-C can cure as well as prevent muscle soreness.*

I could hardly believe what I had seen. Here was a form of C that could provide rapid recovery from as well as prevention of muscle soreness.

When I returned to my home, I asked Dr. Spencer whether ester-C had worked any better than ordinary calcium ascorbate. "Oh, sure, it did," he said. "When such things are chelated, they get into the system faster."

Dr. Spencer's statement was proved at the University of Mississippi. Dr. Anthony Verlangieri, a toxicologist there, tested ester-C in comparison with ascorbic acid in rats and found that ester-C entered the bloodstream in 20 minutes, compared with 40 for ascorbic acid. Dr. Verlangieri also found that ester-C stayed in the tissues longer. Ascorbic acid began leaving the body in urine after 104 minutes; ester-C took 208 minutes to appear in the urine of the rats.

Dr. Jonathan Wright made a similar test on 12 men at the Meridian Valley Clinical Laboratory in Kent, Washington. Dr. Jeffrey Bland expanded the raw-data evaluation. Compared with ascorbic acid 24 hours after ingestion, ester-C left twice as much as vitamin C in the blood serum (liquid component of the blood) and four times as much C in the white blood cells, which store and transport C to sites where it's needed. Only one-third as much ester-C was found in the urine because so much of it remained in the blood and tissues.

Let's go back to Dr. Spencer, however. Obviously, he wasn't treating dogs for exertion-induced muscle soreness in his veterinary practice. He was using vitamin C to treat animals for—are you ready for this? *—arthritis and the effects of hip dysplasia!*

Clarence Schreur, the chemist who discovered ester-C, was searching for something to relieve his wife's arthritis. Schreur's doctor was so impressed that he began manufacturing ester-C. The struggle he fought within his own profession to introduce the new C is a long story. The current owners of the company have continued the fight, and they're gaining ground.

Dr. Charles Dockter tested ester-C on 40 dogs that were suffering from arthritis and/or hip dysplasia. About 75 percent of the dogs experienced pain relief and better mobility in varying degrees. When I met Dr. Dockter during a trip, he confessed that there was one flaw in the test. He had to rely on dog owners, who may not have been faithful in administering ester-C in the correct amounts at the right times. Also, some of the dogs may have been dosed with supplements such as iron that could have tied up part of the ester-C. Dr. Dockter feels that the percentages and degrees of success would have been even higher under a better-controlled program.

Dr. William Jones reported improvement in 50 percent of his human patients who took ester-C and believes that figure could reach 95 percent if they all took it exactly as directed. Dr. Jones was treating a variety of collagen-related ailments, including rheumatoid arthritis.

Two of my own sisters-in-law heard about my research into ester-C and asked to try it to treat arthritis. With one of them, the condition of arthritic fingers was improved. The other had suffered so badly that she couldn't lift her new grandnephew out of his crib. Ester-C solved the problem in a few days.

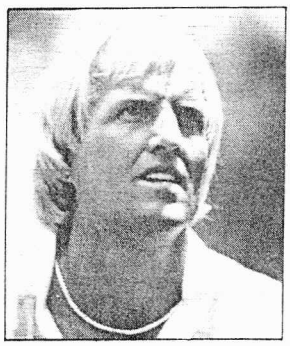
Maintaining endurance in hard-running field trial dogs, I've long thought, is another use for vitamin C, especially ester-C. "It's being done," Gerald Elders told me. "The top-winning Brittany trainer and handler in this country today, Jim Holman of Whittington, Illinois, is using it."

I visited Jim. Scatt Mann, winner of the 1986 National Quail Classic, is always in a dead run from breakaway to pickup. Holman's vet suggested vitamin C to rebuild torn tissues and improve the dog's endurance. It worked, so Jim now gives ester-C to his entire string, which includes winners of the National Pheasant Classic, National Chicken Classic, International All Age Championship and the 1986 All Age Dog of the Year. "Ester-C can't make a dog something he isn't," Holman said, "but being in top condition, plus getting top nutrition, can help him reach the upper limits of his capability."

In the August 1986 issue, I wrote about vitamin C for muscle soreness [illegible] beings. I got a little criticism from readers who suggested that "megadoses" of anything can be dangerous. At some level, they said, everything is toxic. Also, vitamin C, they said, can cause diarrhea and kidney stones.

I asked Gerald Elders to respond to that. Of least importance, he believes, is diarrhea. It's mild if it does occur. In fact, the occurrence of diarrhea is used by some doctors to determine the maximum [illegible] dosage for [illegible] →

# Great Outdoor LEGENDS



GREG NORMAN

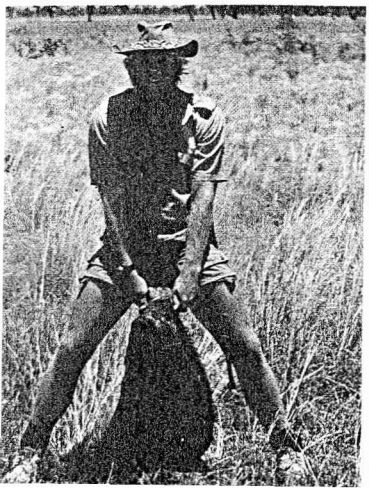
What would Greg Norman be doing today if he were not taking the world of professional golf by storm? "I'd be in the Australian air force, piloting an F-15 or F-16 jet," says Norman, a former cadet and the first golfer to crack the $1 million mark in a single year. (He made $1.2 million in 1986.)

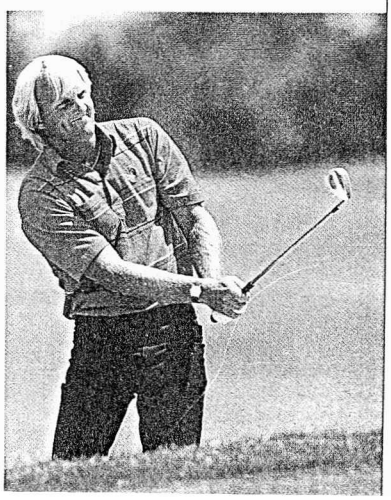
Competing PGA players might wish that Norman, nicknamed the "Great White Shark" for his awesome play in the 1981 Masters tournament, had kept on flying fighters. Although he didn't touch a golf club until age 15, Greg became a scratch player in just 18 months and turned pro at 21. A meteoric rise followed. Last year, in addition to PGA victories at the Las Vegas Invitational and Kemper Open, Norman scored eight wins in Australia and Europe. The Sony Ranking grades him as the finest player in the world today.

When Norman isn't stalking the golf greens, he prowls the Australian Outback on foot in search of wild boar and other game. "I like a physical hunt," he says, "one without dogs or guides that pits my wits against the quarry's." Norman also likes the solitude of fishing. Being in the outdoors eases the pressures of competition and the public spotlight.

How long will the 32-year-old superman of golf stay in the game? "Until I wake up one morning and no longer want to play. Then I'll go hunting or fishing." We salute you, Greg Norman, a great outdoor legend.

Presented by the U.S. Army.





age for individual patients. When diarrhea is reached, they back off the dosage just a bit. You can do the same.

Regarding toxicity, a California lab fed rats a quantity of ester-C that was equivalent to giving a 150-pound man 1,440 tablets, or 720 grams, of C in one day. The rats lived despite the extreme overdose.

Gerald said that ascorbic acid can increase oxalate levels in the urine in a small minority of people whose systems are inclined toward kidney stones, who are magnesium or vitamin B6-deprived, and who are using more than six grams of ascorbic acid daily. Regardless of dosage, normal or average people do not experience abnormal increases of oxalates in the urine when taking C. In addition, the Meridian Valley study found only one-fifth the amount of oxalates in the urine of those on ester-C as compared with those on ascorbic acid. Also, taking *occasional* megadoses to prevent muscle soreness is quite different from taking megadoses continuously.

One doctor thought that my taking 12 grams a day (when hunting all day in the mountains) was a bit foolish, and he quoted a theory that the body only retains 1,500 mg anyhow. I tried cutting down to six grams a day and ended up with strained knee muscles. Obviously, what the body can retain and what it uses under extreme demand are two quite different things.

One thing did puzzle me. Vitamin C is supposed to help fight colds and flu. I caught two colds right after being on 12 grams a day for a while. "You might have had 'rebound scurvy,' " Gerald told me. "Your enzyme activity adjusted to 12 grams. You quit abruptly, and the high number of enzymes then used your body's remaining C right down to deficiency levels. That laid you open to colds. Always taper off from high doses."

For more information about ester-C and how to purchase it, contact Inter-Cal, Dept.OL, 421 Miller Valley Rd., Prescott, AZ 86301 (602-445-8063).

*Autographed copies of Larry Mueller's innovative new book,* Speed Train Your Own Retriever, *are available for $16.95 postpaid from Retriever, Box 143, St. Jacob, IL 62281.*

## Fish Fried

Friday Hvass Nice Day, So Smith Andes Friend Jones Wendt Tew Lake Furr Fish. Bothum Were Eager Fishman. Smith Lyke Bass: Jones Tink Pike Best. Dey Pack Flies, Wurms, Grubbs, Leech, Menne Bates, Creel, Nett, Hooks, Rod, Boots, Mutsch Mohr Gearin Carr.

"Letts Getter Going, Auld Wahlroos," Smith Say. "Fisher Reddy Fore Catchings."

Awn Way, Frenz Made Wager: Furst, Most, Larges Fish. Smith, Aulwes Kidder, Mock Jones Chance.

"Eichen Ketcham Mohr Fast," Smith Bragg. "Heaps Ovall Kinden Waite. Isle Getti Lotti Ure Cash."

Atwater, Both Fishman Cast Fromm Rock. Nutting Bittner Hooks.

"Heck," Jones Whimper. "Letts Baskin Violet Raze. Noe Fisher Takin Holdahl Day. Eye Kvittem!"

Sadd Sack Jones Restad. Jones Gott Sun Berndt. Fisher Smith Cast. Smith Catcher Weed, Break Line.

Poor Tripp.

Beddor Luck Tammaro, Boyce.

*—William J. Hanvik is out to disprove the old saying that the phone book is long on cast but short on plot. In this essay on a fishing contest, he uses only names from the Minneapolis telephone directory. No company names are used—that would be too easy—just individual names of persons. When read aloud, it seems to come out with a Scandinavian accent.*

# The Well-Oiled Dog

*Getting your dog in top condition and keeping him strong and healthy will make him a hunting machine. Here's how.*

By Larry Mueller
HUNTING DOGS EDITOR

Tomorrow holds the consequences of today, and nowhere is that more apparent than with hunting dogs. How we care for our canines in the field today determines how—and if—they will hunt tomorrow.

It's not an exaggeration to say that hunts are only as good as the dogs, so getting your partner in shape and maintaining his health in the field is more than just common sense, it's crucial. Here are some tips that will help.

## Dogaerobics

Ideally, opening day should be prepared for *many* days in advance by getting your dog in shape. Hunting dogs are like athletes. You can't expect your dog to perform well if he's been lazing around the house or kennel all year.

Treadmill tests conducted by Ralston Purina indicate that top-notch conditioning for a dog may take six to eight weeks. During tests of that duration, the Purina dogs' running program increased from 0 to 15 miles a day, five days a week. The dogs' heart rates rose with each increase in effort and eventually plateaued. After that, heart rates stabilized, even as the running distance eventually doubled.

Trainer Bob West conditions dogs for two months using an adjustable running harness made by T.E. Scott (catalog: 9252 Crawfordsville Rd., Indianapolis, IN 46234). West begins by hooking two-pound lengths of heavy logging chain on each side of the dog's harness, then gradually increases them to five or six pounds. The extra weight helps speed aerobic and muscle conditioning.

Swimming is also a great workout, especially when dogs are chasing ducks around a pond. Walking or jogging with you does little for a dog because it doesn't raise the dog's aerobic rate high enough to make gains.

## Vitamin Program

Realistically, ideal conditioning is not always possible to achieve, so it's up to you to compensate. Vitamin C, by preventing extreme muscle soreness, can go a long way toward overcoming lack of fitness.

Unconditioned dogs can be sapped of energy in one day and remain useless for days after. With vitamin C, they keep hunting. I take the vitamin as well, and within about three days, both the dogs and I are hitting our stride. The most effective form of C for dogs is Ester-C, available in health-food stores. I've found that about 3.5 grams for a 50-pound dog is the right dose (more about this amazing vitamin in a later story). Vitamin C is water soluble, so it's entirely safe. The only side effect from too high a dose will be diarrhea, in which case you should cut back the amount.

**A TRAINING HARNESS utilizing logging chain speeds preseason conditioning. Don't forget to bring water (top); teach the dog to accept it directly from the bottle.**

## Water...Water

Water for your dog is obviously critical in the field. In another Purina study, conditioned dogs without extra water ran until exhausted enough to quit on their own. Two weeks later the dogs ran again, but with small amounts of water given to them before and during the run. The water enabled the dogs to run 50 percent longer.

Many hunters, especially during hot, sunny early-season hunts, pack a two-liter bottle of water in their hunting vest. Some people even carry a small bowl, but most train their dogs to accept water poured slowly and directly into the mouth. That's easy to teach during preseason tune-ups and conditioning. Just call in the hot dog, lift a lip, and pour in a small amount of water. Or use a squirt bottle. By opening day, he won't have to be called when he needs a drink.

*continued from page 21*

want a wet dog, too exhausted to even groom himself, to spend a cold (or overly hot) night in the vehicle.

An inventive Texas bird hunter, Leon Measures, has evolved a nearly perfect dog trailer. For one thing the trailer, which is towed behind a vehicle, has built-in compartments in which his dogs' individual carriers fit securely. But Measures' most thoughtful innovation is an air snorkel. It stands high above the trailer to scoop in fresh air that contains neither exhaust nor road dust. A booster blower helps create inside air pressure that further prevents exhaust or dust from entering the trailer.

"Any hunter who rode a mile on a back road in his dog trailer would have a snorkel before the next trip," Measures says.

### Doctoring Your Dog

At lunch and after the hunt, give your dog a quick check. Remove weed seeds from the eyes with water. Pull cactus needles straight out using a hemostat (a surgical form of needlenose pliers that lock closed). Don't try to rub needles out; they'll break off and be impossible to remove. The hemostat works well on sandburs, too. These can be a serious threat if they get under the skin and migrate into the body. Small cuts, such as those made by barbed wire, can be treated with Neosporin ointment, but three-corner tears require stitches by the vet.

There are a variety of time-consuming ways to remove porcupine quills, but so far, I've been very successful by getting there quickly and yanking them out with pliers. I suspect that fooling around too long gets quills broken off. Then they may have to be removed surgically to avoid migration in the body.

Ticks have become a special problem since the spread of Lyme disease, which affects dogs as well as humans. Despite what you've read, do not remove them with a hemostat, tweezers, pliers, or your fingers. These can squeeze the body and force the Lyme-carrying spirochetes out of the tick and into your dog. */continued on page 86*

In severe heat, use some of the water to cool the dog externally, but don't pour it over his back—it'll run right off the fur. Notice that if given the opportunity to cool himself, a dog will drop his chest and belly into water—that's where to splash water with your hand.

### Alternative Water?

Purina studies have also discovered that out-of-shape dogs convert carbohydrates to sugar as their primary energy source. After becoming conditioned, however, fat particles enter the blood as the energy source. That explains why susceptible, unconditioned dogs may suffer from hypoglycemia (low blood sugar) early in the season. Failing to pace themselves, they run to exhaustion, deplete their blood sugar, and collapse.

Veterinarian Scott Green, of Frankfort, Ky., applied common sense to the need for greater staying power afield and invented a product he calls PEP. It's a mix of dextrose, glycine, electrolytes, vitamins, and minerals in water. Vitamin C was also added to prevent muscle soreness. After three years of testing, PEP had prevented hypoglycemia in virtually all the dogs tested, and endurance had risen 85 percent.

Dr. Green recommends that the dogs drink all they can before the hunt, again at noon, and then again during an afternoon break. If that doesn't empty the bottle for a 50- or 60-pound dog, give the balance after the hunt. Some dogs like PEP the first time, some don't. Introduce it well ahead of hunting by adding a little to the water. Keep increasing the amount until the dog is drinking pure PEP. For more information, write to PEP Inc., Box 360344, Birmingham, AL 35236.

### Mobile Home

When traveling with the dog, put him in an airline carrier that he's accustomed to sleeping in. He'll be more content and will have a better first night's sleep. Such a kennel may get your dog inside a motel that doesn't allow canines to roam the rooms, which is an important consideration—Duke may well be soaked to the skin after the hunt. You certainly do not*/continued on page 83*



*continued from page 83*

Two products get under the body for removal without squeezing. One is the Tick Nipper, made by Joslyn Designs, 650 Union Valley Rd., Mahopac, NY 10541 (914-628-0364). The other is Pro-Tick Remedy, made by SCS, Box 573, Stony Point, NY 10980.

### Disciplined Diet

Some well-meaning writers recommend carrying food to fuel the dog during the day. Don't. If you can't stand those pleading eyes at lunchtime, give ole Duke a corner of your sandwich. Feeding just puts an extra digestive burden on your dog at a time when his body is being pushed to the max. Feed him lots of top-quality, nutritionally dense dog food, but wait until after the hunt.

Finally, before feeding the dog after the hunt, cool him down and let him drink all the water he wants. This precaution may prevent bloat and save his life. ♣♣♣

Photo: Tom Nanes

*3rd Prize: Mr. Excitement, from Claudia Presto, Kanab, UT.*

*Runner-up: Jesse, from Lorraine Wilkinson, Riverton, UT.*

*Runner-up: Another Utahan! Razz-E Bauman, Ogden UT.*

# Vitamin C – a conditionally essential nutrient for dogs, cats and horses

Do "complete and balanced" pet foods really provide all the nutrients and, most importantly, the proper levels required for health and vitality? Can a 26 week feeding trial, as prescribed by the American Association of Feed Control Officials (AAFCO), produce enough statistically relevant data to determine if a food fulfills the lifetime nutrient needs of dogs and cats? And, finally, to what extent do stress, age, pregnancy and exposure to chemicals and infectious agents change the nutrient needs of animals? Veterinary nutritionists and clinicians are intensifying their search into the relationship between altered physiological states and macro/micro nutrients, and are better defining "essential" and "conditionally essential" nutrients.

Essential nutrients are necessary for maintaining life and must be provided in the food, since they cannot be synthesized in the body. A chronic deficiency of the amino acid taurine in cats, for example, results in dilated cardiomyopathy, central retinal degeneration and blindness. Without supplemental vitamin C, guinea pigs develop scurvy and eventually die. Other nutrients, however, may become essential only when the normal homeostatic state is upset. Obese "conditionally essential" nutrients can be supplied exogenously or produced endogenously. Vitamin C is a potent antioxidant and is considered by many to be a prime example of a conditionally essential nutrient in dogs, cats and horses.

Vitamin C has excellent antioxidant properties, is an essential component of many cellular biochemical reactions and may protect against free radical-mediated tissue damage. Although dogs, cats and horses (unlike humans and other primates, guinea pigs, fruit bats and red-vented bulbul birds) are capable of producing their own vitamin C, it is uncertain whether the effects produced are enough to counter the effects of stress, disease, exercise, breed predilections to inherited dysfunctions, environmental pollutants and irritants, poor quality foods and age.

Last year the Inter-Cal Corporation of Prescott, Arizona, began providing Best Friends Ester-C, a non-acidic product containing vitamin C, calcium ascorbate and C metabolites. Ester-C helped many of the dogs walk and "feel" better. In fact, in a series of double-blind crossover trials, Ester-C proved twice as effective as ordinary vitamin C in reducing lameness and discomfort and in increasing mobility and activity. Our work confirmed the results of earlier studies by a Norwegian veterinarian who had found that Ester-C improved the mobility of dogs suffering from various joint disorders.

Supplementing the diet of dogs, cats and horses with vitamin C may be a way to reduce free radical damage to tissue and organ systems and prevent, or even reverse, disease. Unfortunately, ordinary vitamin C is an acid and many animals are unable to tolerate the dose necessary to achieve maximum intracellular levels. From our experience at Best Friends, it appears that Ester-C is the best way to provide this important conditionally essential nutrient to pets.

The United States of America

The Commissioner of Patents and Trademarks

*Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.*

*Therefore, this*

United States Patent

*Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.*

*Commissioner of Patents and Trademarks*

*Attest* Melvinia Gary

# United States Patent [19]

**Markham**

[11] **Patent Number: 4,822,816**

[45] **Date of Patent: Apr. 18, 1989**


[54] **COMPOSITIONS AND METHODS FOR ADMINISTERING VITAMIN C**


[75] Inventor: **Richard G. Markham**, Prescott, Ariz.

[73] Assignee: **Oxycal Laboratories, Inc.**, Prescott, Ariz.



[21] Appl. No.: **36,598**

[22] Filed: **Apr. 10, 1987**

[51] **Int. Cl.**[4] ..... **A01N 43/08**; A61K 31/335

[52] **U.S. Cl.** ..... **514/474**

[58] **Field of Search** ..... 514/474, 922, 163, 460, 514/473; 424/10



[56] **References Cited**

U.S. PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| 3,459,863 | 8/1969 | Apelian et al. | 514/474 |
| 3,781,423 | 12/1973 | Aoka et al. | 514/474 |

OTHER PUBLICATIONS

Lewin-Vitamin C its Molecular Biology and Medical Potential, Academic Press (1976).



*Primary Examiner*—Douglas W. Robinson
*Assistant Examiner*—Roger E. Gobrogge
*Attorney, Agent, or Firm*—William H. Drummond



[57] **ABSTRACT**

A composition useful for administering Vitamin C to a subject comprises a compound having Vitamin C activity and at least one compound selected from the class consisting of the aldono-lactones and edible salts of L-threonic, L-xylonic and L-lyxonic acids.

High Vitamin C levels in the human body are established by orally administering to a human subject an effective amount of this composition.


**2 Claims, No Drawings**

UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION

PATENT NO. : 4,822,816

DATED : April 18, 1989

INVENTOR(S) : Richard G. Markham

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, line 28, delete "derivations" and insert "derivatives"

Column 1, line 50, delete "derivations" and insert "derivatives"

Column 1, line 59, delete "on" and insert "only"

Column 2, line 49, delete "produce" and insert "product"

Column 3, line 28, delete "pump-drying" and insert "pumping-drying"

Column 3, line 55, delete "take" and insert "taken"

Column 4, line 11, delete "MB" and insert "MG"

Column 4, line 38, delete "text" and insert "test"

Column 5, line 46, delete "produce" and insert "product"

Column 7, line 22, delete "also-lactone" and insert "aldo-lactone

Signed and Sealed this

Sixteenth Day of January, 1990

Attest:

JEFFREY M. SAMUELS

*Attesting Officer*

*Acting Commissioner of Patents and Trademarks*

# COMPOSITIONS AND METHODS FOR ADMINISTERING VITAMIN C

This invention relates to an improved form of Vitamin C.

In another respect, the invention concerns improved methods for establishing Vitamin C levels in the human body.

In yet another respect, the invention pertains to methods for improving the human body tolerance to Vitamin C.

In still another respect, the invention relates to a Vitamin C derivative composition which is more effectively absorbed and retained in the human body.

Prior workers have identified over 300 separate metabolic mechanisms in which Vitamin C is involved in physiologic reactions. These mechanisms range from the antiscorbutic effect first observed by Dr. Robert Lind in 1740 to more recently discovered anti-oxidant free-radical scavenging reactions, the co-reaction with enzymes in the formation of collagen, energy metabolism accentuation in the polynuclearleucosites and facilitation of iron absorption.

The clinical effects of such metalbolic reactions have been widely recognized and reported. For example, the free-radical scavenging effect is believed to enable the body to convert carcinogens to non-toxic derivations which are eliminated in the urine and, consequently, to ameliorate the effects of smoking and exposure of the body to other environmental pollutants. Animal studies have demonstrated that body enzymes convert ascorbates to oxidation products which have demonstrated tumor growth inhibition.

Consequently, there is little scientific doubt that the establishment and maintenance of effective levels of Vitamin C and its derivatives in the human body yield important health advantages. The presence of Vitamin C in substantial concentration has been observed in the adrenals, ovaries, brain, pituitaries, liver, spleen, blood cells, blood serum, and extracellular lung liquids.

Most animals have a liver enzyme which enables them to actually mantufacture Vitamin C in situ by conversion of blood sugar into ascorbic acid. However, humans do not have this enzymes. As a consequence, the Vitamin C which is required by the human body for the various metabolic reactions discussed above must be ingested with the human diet. Futhermore, the human body does not have the ability to store Vitamin C—if unmetabolized, it is excreted. Low levels of Vitamin C and its derivations in the human body produce a variety of undesirable physiological responses and extremely low levels produce extreme responses which may result in death, e.g., from scruvy. Wholly apart from these "normal" requirements of Vitamin C, it is important in some therapeutic modalities to establish and maintain above-normal Vitamin C levels in the body. These above-normal concentrations are difficult to establish and maintain because the human body exhibits on a finite tolerance for Vitamin C, with resultant diarrhea and other side reactions, such as gastric irritation and inflammation if these tolerances are exceeded.

I have now discovered compositions and methods for improving the establishment and maintenance of high levels of Vitamin C (including its derivatives) in the human body. Briefly, the composition which I have discovered comprises a compound having Vitamin C activity and at least one compound selected from the



class consisting of the aldono-lactones and edible salts of L-threonic, L-xylonic and L- lyxonic acids.

A method which I have discovered for establishing Vitamin C levels in the human body includes the step of orally administering this composition to a subject.

Another method of the invention comprises the step of converting L-ascorbic acid to this composition and orally administering this composition to the subject.

As used herein, the term "compound having Vitamin C activity" means Vitamin C (L-ascorbic acid) and any derivative thereof which exhibits ascorbitic activity as determined by the standard iodine titration test. Such derivatives include, for example, oxidation products such as dehydroascorbic acid and edible salts of ascorbic acid such as, illustratively, calcium, sodium, magnesium, potassium and zinc ascorbates.

The metabolites of ascorbic acid and its derivatives include the aldo-lactones and edible salts of aldonic acids. As will appear, the compositions of the present invention are characterized by the presence of at least one or more of these metabolites corresponding to three specific aldonic acids: L-threonic acid, L-xylonic acid and L-lyxonic acid.

The presence of one or more of these metabolites in the compositions of the invention is both a convenient way of identifying such compositions and is also necessary to achieve the desired result, improvement in Vitamin C absorption and/or retention.

The component of the compositions of the invention which, as indicated above, has "Vitamin C activity" can be L-ascorbic acid or any of its derivatives which have the ability to provide the ascorbic/ascorbate moiety in physiologic processes, e.g., dehydroascorbic acid, calcium ascorbate which are most commonly employed in Vitamin C food supplements, as well as a wide variety of other edible (nontoxic) salts such as potassium, sodium, magnesium and zinc ascorbate. In general, any such compound which exhibits C-vitaminic activity will suffice and this can be conveniently determined by the well-known standard "iodine test".

A suitable method for preparing the compositions of the invention comprises reacting L-ascorbic acid with a non-toxic metal compound, e.g., calcium carbonate, sodium bicarbonate, under oxidizing conditions at an elevated temperature, e.g., 40° C.-98° C., to convert a substantial proportion of the ascorbic acid to its corresponding salt, e.g., calcium or sodium ascorbate, and drying the reaction mixture to produce a solid produce of essentially neutral pH (e.g., 6.0-7.2). Preferably, a slight stoichiometric excess of the metal salt reactant is provided. The resultant product has an iodine ascorbate activity in the range 100-400 mg./500 mg. sample with the higher activity preferred for practical reasons. Longer heating at oxidizing conditions produces lower iodine ascorbate activity.

The compositions of the invention appear useful in administering Vitamin C to patients who have low ascorbic acid tolerance. In particular, paatients who have a tendency to form kidney stones are particularly susceptible to difficulties when ingesting ascorbic acid and its common derivatives, calcium ascorbate, which cause elevated urine oxalate levels. It has now, however, been discovered that the compositions of the present invention can be administered without increasing the oxalate level in the urine to the levels encountered when the prior art compositions are ingested. Therefore, the present compositions and methods are especially suitable as

a means of establishing and maintaining ascorbate body levels in such kidney stone-prone subjects.

The following examples are presented for purposes of illustrating the practice of the invention and are not intended as limitations on the scope thereof.

## EXAMPLE 1

To an 80 gallon, steam-heated stainless steel reaction vessel was added 60 lbs. of hot (44° C.) water.

Ascorbic acid-U.S.P., 110.23 lbs., was added in one portion to the hot water. The resulting slurry was mechanically stirred and heated with steam (pressure 15 p.s.i.) until a temperature of 70° C. was achieved.

To the aqueous slurry of ascorbic acid was added 23 lbs. of calcium carbonate. The incremental addition of the carbonate required 3–4 minutes. The reaction mixture appeared gray in color and much foaming due to the evolution of $CO_2$ was evident.

After eight minutes of stirring, most of the foaming had subsided and the reaction mixture appeared red-brown in color. The reaction temperature was 80° C.

Stirring and heating were continued for 15 minutes until the temperature of the reaction mixture reached 98° C. where it was maintained for an additional 20 minutes, during which an additional 8.25 lbs. of calcium carbonate was added, with stirring. The reaction mixture was then pumped to a double-drum steam-heated dryer (surface temperature 250° F.). The pump-drying step required 35 minutes. The dried product was light-tan color and the yield was approximately 120 pounds of product.

Assays were performed immediately on 5.00 g. samples dissolved in 500 ml. distilled water.

The material collected during the drying process showed 400 mg. ascorbate activity per 500 mg. by the standard iodine titration technique. The same aqueous solution showed pH 7.0.

## EXAMPLE 2

The following example describes clinical tests comparing the product of Example 1 (test) with L-ascorbic acid and citric acid (placebo), measuring intracellular ascorbate levels, urinary ascorbate output and urinary oxalate excretion at various times after ingestion of standard doses of the test, L-ascorbic acid and placebo.

### Summary of the Protocol

Twelve men, agaes 27 to 45, were studied.

All were instructed that they should be on a low Vitamin C diet for one week prior to the study. (No citrus products and no large amounts of green leafy vegetables).

Following overnight fast, blood and 24-hour urine samples were take. White blood cell and 24-hour urinary ascorbate and oxalate levels were determined and correlated with serum ascorbate levels.

The 12 men were divided into three groups, and were given the following supplements:

(a) Test Group: 4000 MG* per day of the product Ex. 1. *4000 MG is equivalent in ascorbate activity (iodine test) to 3000 MG L-ascorbic acid.

(b) Ascorbate Group: 3000 MG of L-ascorbic per day.

(c) Citric Acid Group: 3000 MG of citric acid per day.

All 12 continued on the low Vitamin C diet. Blood samples were taken at 0, 4, 8 and 24 hours after morning ingestion of the designated supplements. Urinary 24-hour ascorbate and oxalate levels were determined.

After a wash-out period (varying from two days to several days, due to job situation of the participants), the groups were switched to another supplement, as follows:

(a) Test group to citrate group.

(b) Citrate group to ascorbate group.

(c) Ascorbate group to test group.

Supplements were taken at the same level (4000 MG of EX. 1 product, 3000 MB of L-ascorbic and 3000 MG of citric acid) by all three groups. Blood samples were again drawn at 0, 4, 8 and 24 hours from time of ingestion. A 24-hour urine was also collected by all 12 participants at the end of the period. Again, all specimens were analyzed for their respective concentration of ascorbate and oxalate level.

### Analytical Procedures

The analytical procedures utilized are described in:

*Clinical Chemistry, Principles and Techniques,* edited by Richard J. Henry, Donald D. Cannon and James W. Windelman, Harper and Row, 1974, p. 1393–1398.

*Standard Methods of Clinical Chemistry,* J. S. Roe, edited by Seligson D. New York, Academic Press, 1961, Vol. 3, p. 35.

In the quantitation of 24-hour urine oxalate, an aliquot of urine is shaken with an adsorbent which selectively binds the oxalate. The extracted urine is discarded and the oxalate is eluted from the adsorbent with dilute alkali.

Oxalate is oxidized to hydrogen peroxide and carbon dioxide by oxalate oxidase. The hydrogen peroxide reacts with 3-methyl-2-benzothiozolinone hydrazone (MBTH) and 3(dimenthylamino)benzoic acid (DMAB) in the presence of peroxidase to yield an indamine dye with a maximum absorbance at 590 NM.

The urine oxalate text is further described in the following references:

Hodgekinson, A.: *Oxalic Acid in Viology and Medicine,* Academic Press, New York, 1977.

Robertson, W. D.; Rutherford, A.: *Aspects of the Analysis of Oxalate in Urine,* Scan J. Uro Nephrol, Suppl. 53, pg. 85, 1979.

Lamden, M. P.; Chrystowski, G. A.: *Urinary Oxalate Excretion by Main Following Ascorbine Acid Ingestion,* Prog. Soc. Exp. Biol. Med. 85:190, 1954.

Costello, J.: *The Effect of Ascorbic Acid on Oxalate Metabolism in Human Biochemistry and Clinical Pathology,* edited by G. A. Rose, W. G. Robertson and R. W. E. Watts. Proceedings of an International Meeting in London, 1971, pp. 270–273.

The results of this clinical study are set forth below:

TABLE 1

| | Percent Change* in Group | | |
|---|---|---|---|
| | Citrate | L-ascorbic | Test |
| Serum Ascorbate Level | | | |
| 4th Hour | 10 | 180.3 | 264.8 |
| 8th Hour | 19.6 | 91.6 | 144.2 |
| 24th Hour | 5.9 | 24.6 | 56.2 |
| 7th Day | | 45.3 | 102.5 |
| White Blood Cell (WBC) Ascorbate Level | | | |
| 4th Hour | −40.5 | 34.1 | 38 |
| 8th Hour | −21.2 | −21.9 | −6.80 |
| 24th Hour | −6.3 | −5.3 | 18.2 |
| 7th Day | | 27.6 | 30.5 |

TABLE 1-continued

| | Percent Change* in Group | | |
|---|---|---|---|
| | Citrate | L-ascorbic | Test |
| 24th Hour Urine Ascorbate | | | |
| % Change | 27.7 | 2760.6 | 486.3 |
| MG/24 Hour | 43.65 | 314.5 | .252 |
| 7th Day Urine Ascorbate | | | |
| % Change | | 4583 | 617 |
| MG/24 Hour | | 459 | 321 |
| 24th Hour Urine Oxalate | | | |
| % Change | 7 | 162 | 35.9 |
| MG/24 Hr. | 25.9 | 63.8 | 41.6 |

*increase unless otherwise indicated.

The conclusions which were drawn from this study are:

Serum Ascorbate Level:

At 4, 8 and 24 hours and 7 days later, test groups had higher serum ascorbate level as compared to both the citrate group and the L-ascorbic group.

WBC Ascorbate Level:

Although all 8th hour WBC ascorbate groups showed an average decrease, test group had the smallest percentage decrease. Four and 24-hour measurement plus the 7th day level showed that test group was able to maintain the highest white blood cell ascorbate level.

24-Hour WBC Ascorbate:

24-hour post various loadings of: citrate, L-ascorbic and test produce --

Both the citrate group and L-ascorbic group showed a decrease in WBC ascorbate levels. Test group maintained a much higher level compared to baseline.

7 Days Post Loading of L-Ascorbic and Test:

Average percentage change in WBC Ascorbate is still higher in the Test Group than in the L-ascorbic group.

24-Hour Urine Ascorbate Output:

Both in average percentage change and in absolute total means values, the test groups had less ascorbate output than L-ascorbic.

7 Days - 24 Hour Urinary Ascorbate Output:

Test groups have less ascorbate output than the citrate and the L-ascorbic groups.

24-Hour Urinary Oxalate Output:

Oxalate output is greatly decreased in the test group as compared to the ascorbic group. This means that while taking test produce as a supplement, a person has less chance of forming oxalate-containing kidney calculi than a person taking L-ascorbic.

7 Day 24 Hour Oxalate Output:

Prolonged supplementation with test product leads to less excretion of urinary oxalate than supplementation with L-ascorbic.

## EXAMPLE 3

To a 2-liter, reaction vessel equipped with an agitator and a thermometer is added 300 ml distilled water and 440 g (2.5 moles) L-ascorbic acid. To this stirred slurry, finely divided calcium carbonate is added incrementally at a rate such as to produce a constant evolution of carbon dioxide (reaction byproduct), the reaction temperature being maintained at about 20° C. The addition of calcium carbonate is suspended after about 25 g to 37.5 g have been added (representing from about 20% to 30% of that required for complete reaction with the L-ascorbic acid charge).

At this point, the temperature is raised to 80° C. Further additions of calcium carbonate are begun, the temperature being maintained in the range 60° C. to about 70° C. The total quantity of calcium carbonate added is 125 g (1.25 moles).

The reaction mixture is transferred to a shallow container maintained at a temperature of between 60° C. and 80° C., for a period of from 12 to 36 hours, during which time the pH of the mixture rises to a pH range of 6.0–7.0. At this point, the excess water is removed under vacuum.

The dry products are light tan in color and readily soluble in water, except for unreacted calcium carbonate, to produce neutral solutions.

## EXAMPLE 4

Clinical studies using the product of Example 3 yield similar results to those set forth in Example 2.

## EXAMPLE 5

The products of Examples 1 and 3 are subjected to qualitative analysis, as follows:

Ascorbic acid, calcium ascorbate and dehydroascorbic acid were separated from the product by chromatography and the residue was subjected to nuclear magnetic resonance spectroscopy. Likely possibilities for the structures of the components detected by spectroscopy were formulated and these authentic compounds were then synthesized. After nmr spectra of these authentic compounds were obtained, they were compared to the nmr spectra of the test specimens. A match of the spectra was used to identify the components of the test specimens.

The techniques employed were $^{1}H$ and $^{13}C$ nmr. The aldonic acid salts identified are the calcium salts of L-threonic acid, L-xylonic acid and L-lyxonic acid.

## EXAMPLE 6

The procedures of Example 1 are repeated except that the reactant added to the ascorbic acid is changed to yield correspondingly different edible salts of ascorbic acid.

| Reactant | Salt |
|---|---|
| sodium bicarbonate | sodium ascorbate |
| magnesium carbonate | magnexium ascorbate |
| potassium bicarbonate | potassium ascorbate |
| zinc oxide | zinc ascorbate |

These products produce similar physiological results to those described in Example 2 and contain the aldonic acid salts corresponding to those identified in Example 5.

## EXAMPLE 7

Quantitative analysis of the products of Examples 1, 4 and 6 is performed. The products have the indicated compositions:

| | Wt. % |
|---|---|
| Anhydrous metal Ascorbate | 80–82 |
| Unreacted metal reagent | 6–7 |
| Dehydroascorbic Acid | 3–8 |
| Moisture | 1.5—3 |
| Aldonic Acid Derivatives | 5–6 |

The aldonic acid derivatives include derivatives of the indicated acid in the following approximate proportions:

| Acid (Derivative) | Parts by Weight |
|---|---|
| Threonic | 8 |
| Xylonic | 3 |
| Lyxonic | 1 |

There are indications that one or more of these aldonic acids may be partially linked.

## EXAMPLE 8

Animal feeding studies of the product of Example 1 provide similar results to the human studies of Example 2.

## EXAMPLE 9

The procedure of Example 1 is repeated except that no metal compound reactant (e.g., calcium carbonate) is added. The ascorbic acid is, however, subjected to the same oxidizing, heating, drying steps. The product consists of anhydrous ascorbic acid product, DHA water and also-lactone derivatives of threonic, xylonic and lyxonic acids. This product yields similar physiological results to those described in Example 8.

## EXAMPLE 10

The procedures of Example 2 are repeated except that the test product is synthesized by mixing reagent grade calcium ascorbate with

Test A—Threonic Acid (calcium salt)

Test B—Xylonic Acid (calcium salt)

Test C—Lyxonic Acid (calcium salt)

in the same weight proportions as the components are found in Example 7.

The tests of Example 2 are repeated using these test compounds and using pure calcium ascorbate as an additional control.

These tests confirm that the physiological activity of the mixed ascorbate-aldonic product is due to the aldonic component and that any one of these aldonic components causes the similarly improved absorption and retention of the Vitamin C component.

Having described my invention in such terms as to enable those skilled in the art to understand and practice it, and having identified the presently preferred embodiments thereof, I claim:

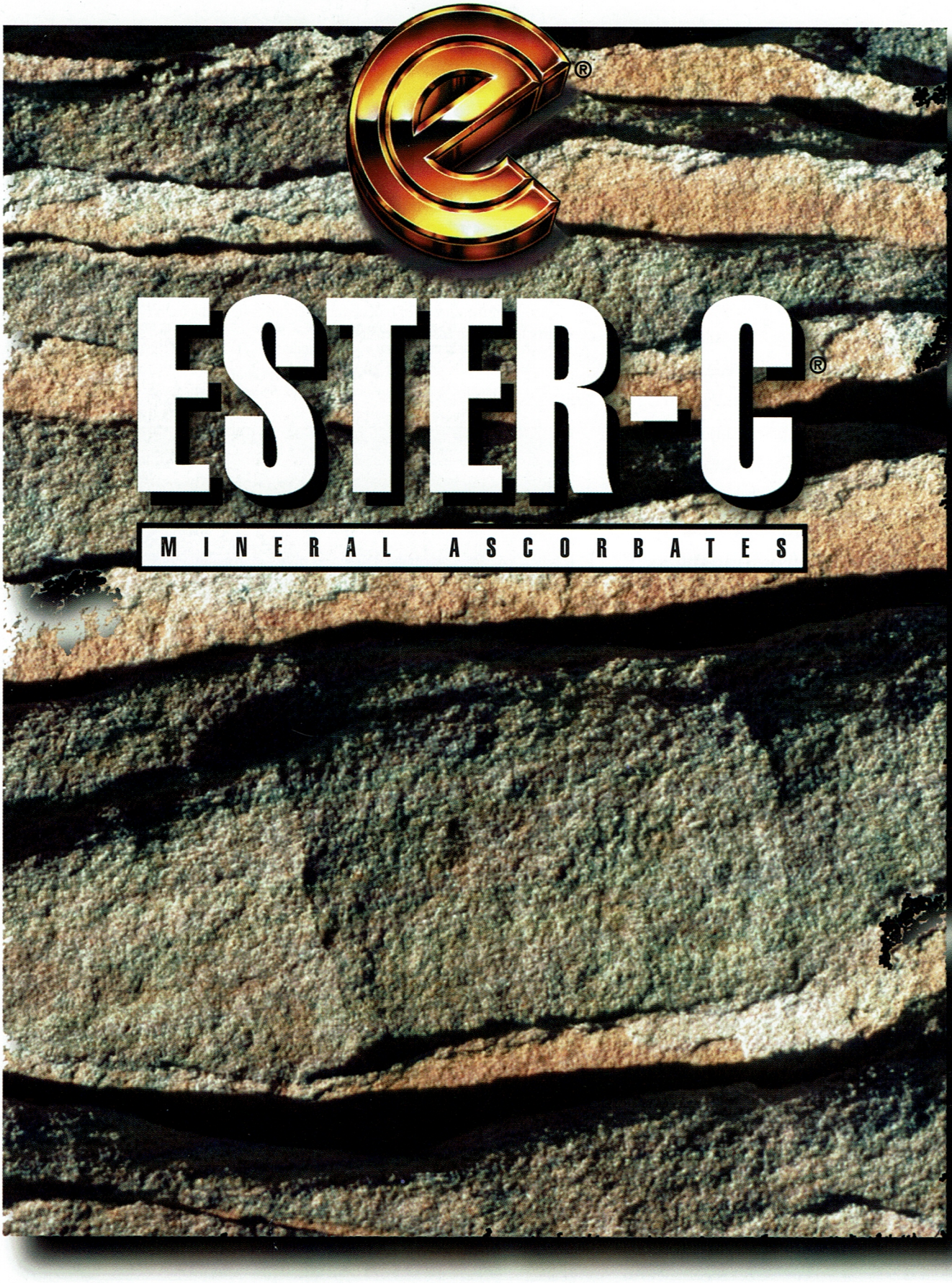
1. A vitamin composition comprising:

(a) an effective amount of a compound having Vitamin C activity, selected from the group consisting of (i) dehydroascorbic acid, (ii) L-ascorbic acid and the edible salts thereof; and

(b) at least one compound selected from the group consisting of the aldono-lactones of L-threonic acid, L-xylonic acid, L-lyxonic acid and the edible salts of L-threonic acid, L-xylonic acid and L-lyxonic acids, in an amount effective to increase the human body absorption rate of said Vitamin C compound.

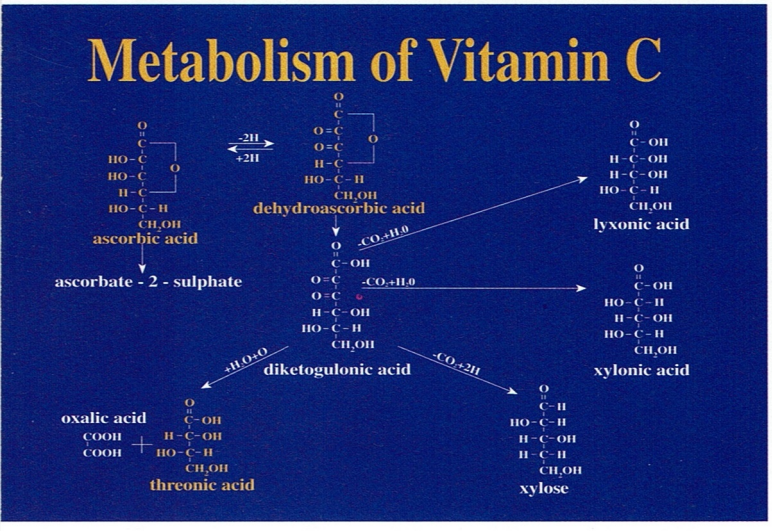
2. A method for establishing high Vitamin C levels in the human body which comprise the step of orally administering to a subject an effective amount of the composition of claim 1.

* * * * *




# RESEARCH SUMMARY

The **need for Vitamin C** has never been greater. Stress, environmental pollution, age and health, as well as **biochemical individuality**, all help to dictate our daily requirement for this essential nutrient. Unlike most other animals, humans cannot make their own Vitamin C. With Vitamin C being part of more than **300 bodily functions**, life itself depends on daily replenishment. Is there a **best form** of this vital nutrient?




# VITAMIN C BIOCHEMISTRY

The profound biological effects of high doses of Vitamin C have led many researchers and health professionals to appreciate that large doses of Vitamin C behave differently in the body than smaller, nutritional doses. Convincing arguments have recently been presented for raising the dietary intake of Vitamin C above present recommended levels to amounts that promote optimal function of the dozens of biochemical reactions in which ascorbate is known to participate as an essential co-factor.

### Vitamin C Metabolites

The body changes Vitamin C into numerous metabolites that may have physiological actions that are different from those of Vitamin C itself. And these metabolites—whether manufactured in the body or ingested along with supplemental Vitamin C—may influence how the vitamin itself is transported and utilized. Dehydroascorbate—the oxidized and transportable form of ascorbate—may be "inactivated" by hydrolysis (i.e., delactonization) to 2,3-diketogulonate . Dehydroascorbate, via the diketogulonate intermediate, may also be degraded both enzymatically and non-enzymatically in many tissues into smaller fragments, such as oxalate and aldonic acids. The level of these metabolites in the body may not rise significantly, however, until very large doses of Vitamin C are administered.

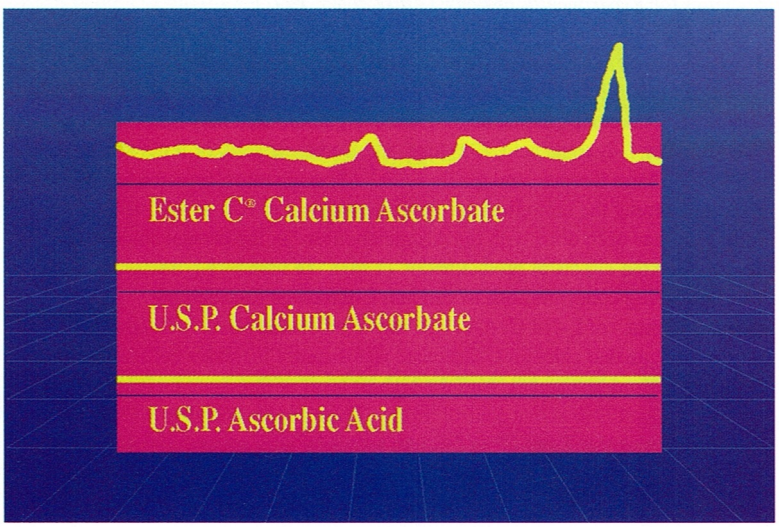
Both the reduced and oxidized forms of ascorbate possess Vitamin C activity, because both forms are interchangeable in the body. They are metabolized quite differently in biological systems, however. Compared to the reduced ascorbate, the oxidized form—dehydroascorbate—is chemically unstable and readily converted to diketogulonate, which possesses no Vitamin C activity. Diketogulonate can be broken down into a variety of smaller fragments by many cells. Chief among these metabolites are oxalate and threonate. Some metabolites are excreted, while others appear to remain in the body and perform other functions.

Threonic acid and other aldonic acids profoundly influence Vitamin C uptake and utilization. The studies of Dr. Anthony Verlangieri, Professor of Pharmacology and Toxicology at the University of Mississippi, have laid the groundwork for our better understanding of how these metabolites interact with Vitamin C in the body. His work has shown that C metabolites—especially threonate—are directly involved with increased blood levels of Vitamin C. Verlangieri and his colleague, Dr. Michael Fay, have used isolated cells in culture to model how Vitamin C is utilized by various body tissues. They have used cells from labora-tory animals as well as humans to study influences on Vitamin C utilization.

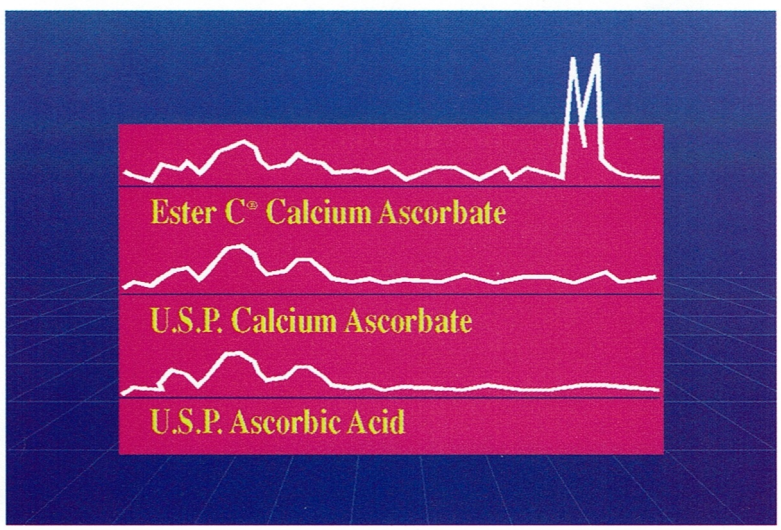
In a number of careful experiments, they observed that exposing cells to calcium threonate increased the uptake of ascorbic acid by these cells. Other aldonic acids were shown to have a similar effect. Verlangieri's group had previously shown that glucose and ascorbate probably share a common transport system for shuttling these molecules through the membrane into cells. This transport mechanism is thought to involve membrane-bound receptors for ascorbate. These lines of evidence strongly suggest that aldonic acids facilitate cellular uptake of ascorbate by influencing cell membrane receptors.

# THE KEY TO THE POWER OF ESTER-C®
BRAND

The only negative side-effect of regular large doses of Vitamin C seems to be gastrointestinal discomfort and diarrhea. These effects are due in large part to the acidity of ascorbic acid itself. A new technology has been developed to manufacture a Vitamin C product that circumvents many of these problems. At the same time, this new process introduces an important metabolite of Vitamin C so it is present along with ascorbate when the supplement is taken. The Inter-Cal Corporation utilizes a unique manufacturing process that neutralizes the acidity of ascorbic acid and maximizes the formation of C metabolites, so they can work synergistically to speed the absorption and utilization of Vitamin C.



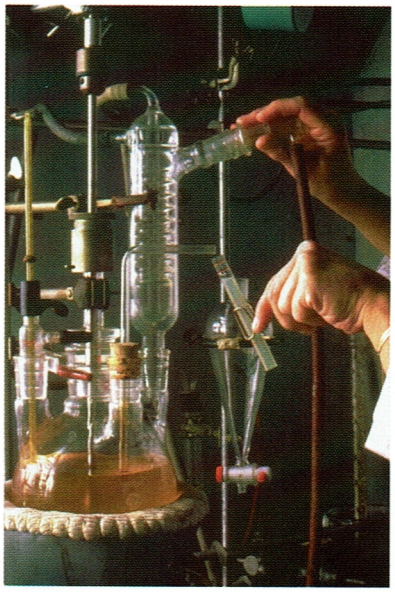

*These graphs are from actual identical segments of Nuclear Magnetic Resonance (NMR) and High Performance Liquid Chromotography (HPLC) analyses of U.S.P. ascorbic acid, U.S.P. calcium ascorbate and* ***Ester-C® Calcium Ascorbate****. NMR and HPLC are "fingerprints" of the chemical make-up of a compound, and the height of the peaks at specific points indicates the amount of a certain material present in a mixture. These graphs are very similiar until the point at the far right is reached. This area indicates the amount of the natural C metabolite, threonate, in each form of Vitamin C.*




**Ester-C®** ascorbate, a revolutionary form of **Vitamin C**, is the most bioavailable form ever developed. **Metabolites** in Ester-C® ascorbate help the vitamin get into cells more efficiently.

Conventional mineral ascorbates are manufactured using either alcohol or acetone. During our exclusive **water-based manufacturing process**, specifically controlled amounts of the Vitamin C in the Ester-C® ascorbate undergo **structural changes similar to those that happen in the body** to produce bioavailable metabolites.

As the graph indicates, Ester-C® calcium ascorbate contains a significant level of the natural C metabolite **threonate**, compared with other Vitamin C supplements.



**Metabolites** are the key to enhancing blood levels and tissue concentration of Vitamin C. **Threonate**, which is the dominant metabolite in the Ester-C® complex, is **body-ready** for these roles in each of the various Ester-C® mineral ascorbates.

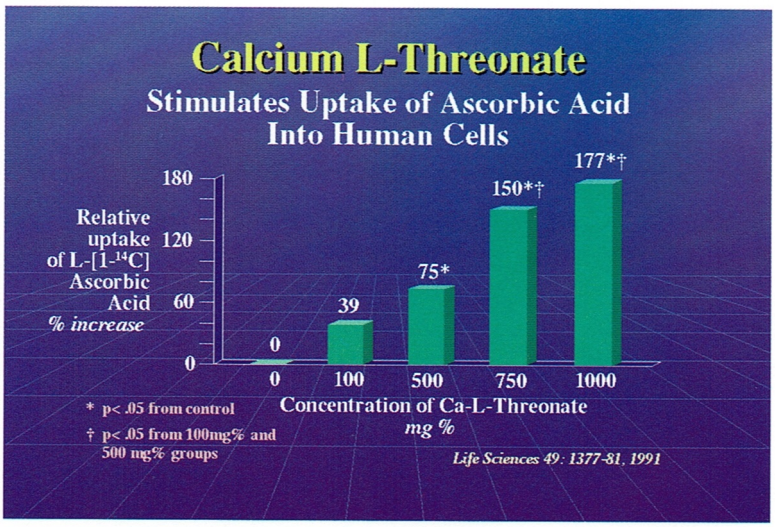
# THREONATE ACCELERATES VITAMIN C UPTAKE

## Stimulating Action of Calcium L-Threonate on Ascorbic Acid Uptake by a Human T-Lymphoma Cell Line

*By M.J. Fay and A.J. Verlangieri*
Department of Pharmacology, School of Pharmacy, University of Mississippi

***Life Sciences, Vol. 49, pp.1377-1381, 1991***
*The following abstract was presented at a meeting of the Federation of American Societies for Experimental Biology (April 21-25, 1991).*

The metabolism of ascorbic acid (AA) involves the oxidation of AA to dehydroascorbic acid which subsequently undergoes a hydrolytic ring cleavage to form 2, 3-diketo-1-gulonic acid. Diketo-1-gulonic acid may become decarboxylated forming L-lyxonic acid, L-xylose, and L-xylonic acid, or it may be further oxidized to form threonic and oxalic acids. Although much research has been done concerning the biological and pharmacological effects of AA, very little work has been done with the AA metabolites. In the present studies we investigated the effects of preincubation (1 hour) of human T-lymphoma cells with increasing concentrations of calcium L-threonate (0-1,000 mg %) on the uptake (30 min.) of L-[14 C}AA (1.25 mg % unlabeled / 0.125µCi labeled). The results indicate that calcium L-threonate significantly increases cellular AA uptake in a dose dependent manner, with the highest level of threonate increasing uptake approximately 177% above control levels. These results correlate with *in vivo* studies showing that Ester-C® (a calcium ascorbate supplement containing AA metabolites) is absorbed more rapidly than AA. The facilitation of cellular AA uptake and subsequent increase in cellular free radical scavenging potential may have important clinical therapeutic implications.



# EFFECT OF ALDONIC ACIDS ON THE UPTAKE OF ASCORBIC ACID

## Effect of Aldonic Acids on the Uptake of Ascorbic Acid by 3T3 Mouse Fibroblasts and Human T Lymphoma Cells

*By M.J. Fay[1], Marilyn J. Bush[2], and Anthony J. Verlangieri[2]*

[1]Department of Physiology, Dartmouth Medical School, [2]Department of Pharmacology, School of Pharmacy, University of Mississippi

*General Pharmacology, Vol. 25, Number 7, pp.1465-1469, November 1994*

This study is strong support for a **biochemical mechanism** involving **threonate** in **cellular uptake** of ascorbate at the level of the **cell membrane**.

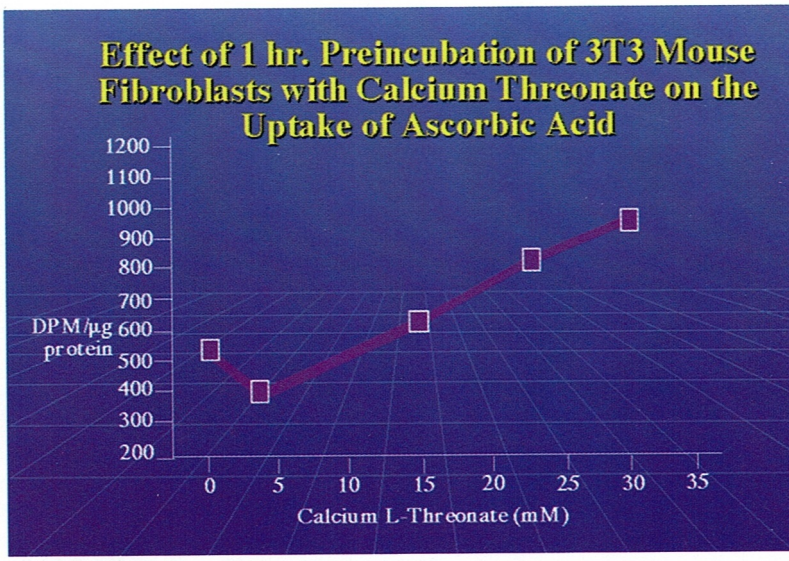
**REVIEW**

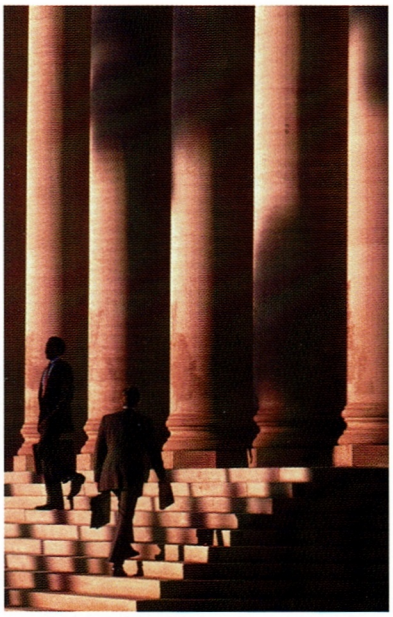
*This paper extends the previous work of Fay & Verlangieri (Stimulatory Action of Calcium L-Threonate on Ascorbic Acid Uptake by a Human T-Lymphoma Cell Line, Life Sciences 49: 1377-1381 (1991)), which reported the potentiating effect of threonate on ascorbate uptake by human T-lymphoma cells in tissue culture.*

*For the work published in General Pharmacology, Fay, Bush, and Verlangieri not only replicated their findings for human T-lymphoma cells (T-cells), but they also extended their research to include a muscle-cell type represented by 3T3 mouse fibroblasts. They found that preincubation of mouse fibroblasts with calcium L-threonate resulted in a dose-related augmentation in uptake of ascorbic acid as compared to non-treated control cell cultures. This result was similar to that seen with the T-cells. This effect was highly statistically significant.*

*L-lyxonate, another Vitamin C aldonic acid metabolite, also significantly increased ascorbic acid uptake in human T-cells, but not in mouse fibroblasts. In the presence of 30.5 mM L-lyxonate, human T-cells incorporated approximately 32,000 dpm (disintegrations per minute) of radioactively labeled ascorbic acid, compared to approximately 11,000 dpm in unsupplemented cells. This ratio represents a 2.9-fold increase in ascorbate uptake due to the presence of lyxonate.*

*Inhibitors of cell membrane transport, such as ouabain and dinitrophenol, reduced ascorbic acid uptake and prevented threonate augmentation of ascorbic acid uptake by human T-cells. The latter result is strong support for a biochemical mechanism involving threonate in cellular uptake of ascorbate at the level of the cell membrane.*





# THE SUPERIORITY OF ESTER-C®'S UNIQUE NUTRIENT FORM

Based on the strength of the research confirming that **metabolites enhance bioavailability and absorption** of Vitamin C, on April 18, 1989, the United States Department of Commerce Patent and Trademark Office issued patent No. 4,822,816, which recognizes the uniqueness of this product.

**United States Patent** [19]
Markham

[11] Patent Number: 4,822,816
[45] Date of Patent: Apr. 18, 1989

[54] COMPOSITIONS AND METHODS FOR ADMINISTERING VITAMIN C

[75] Inventor: Richard G. Markham, Prescott, Ariz.

[73] Assignee: Oxycal Laboratories, Inc., Prescott, Ariz.

[21] Appl. No.: 36,598

[22] Filed: Apr. 10, 1987

[51] Int. Cl.[4] .....A01N 43/08; A61K 31/335

[52] U.S. Cl. .....514/474

[58] Field of Search.....514/474, 922, 163, 460, 514/473; 424/10

[56] References Cited
U.S. PATENT DOCUMENTS

3,459,863 8/1969 Apelian et al. .....514/474
3,781,423 12/1973 Aoka et al. .....514/474

OTHER PUBLICATIONS

Lewin-Vitamin C its Molecular Biology and Medical Potential, Academic Press (1976)

*Primary Examiner*- Douglas W. Robinson
*Assistant Examiner*- Roger E. Gobrogge
*Attorney, Agent, or Firm*- William H. Drummond

[57] ABSTRACT

A composition useful for administering Vitamin C to a subject comprises a compound having Vitamin C activity and at least one compound selected from the class consisting of the aldono-lactones and edible salts of L-threonic, L-xylonic and L-lyxonic acids.

High Vitamin C levels in the human body are established by orally administering to a human subject an effective amount of this composition.

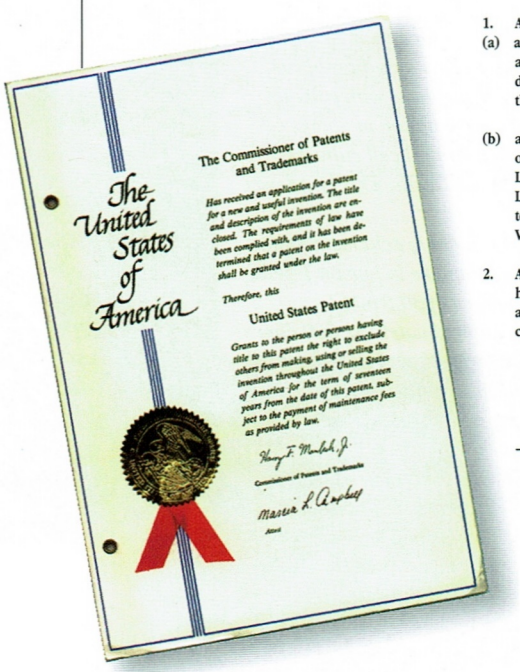
2 Claims, No Drawings

PATENT CLAIMS ALLOWED

1. A vitamin composition comprising:
(a) an effective amount of a compound having Vitamin C activity, selected from the group consisting of (i) dehydroascorbic acid, (ii) L-ascorbic acid and the edible salts thereof, and

(b) at least one compound selected from the group consisting of the aldono-lactones of L-threonic acid, L-xylonic acid, L-lyxonic acid and the edible salts of L-threonic acid, L-xylonic acid and L-lyxonic acids, in an amount effective to increase the human body absorption rate of said Vitamin C compound.

2. A method for establishing high Vitamin C levels in the human body which comprise the step of orally administering to a subject an effective amount of the composition of claim 1.


The United States of America
The Commissioner of Patents and Trademarks
Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.
Therefore, this
United States Patent
Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

# ANTI-SCORBUTIC ACTIVITY OF L-ASCORBIC ACID & ESTER-C®

## Comparison of the Anti-Scorbutic Activity of L-Ascorbic Acid and Ester-C® in the Non-Ascorbic Synthesizing Osteogenic Disorder Shionogi (ODS) Rat

*By A.J. Verlangieri, Ph.D., Michael J. Fay and Anthony W. Bannon*
**Atherosclerosis Research Laboratories, Department of Pharmacology University of Mississippi, University, MS 38677**

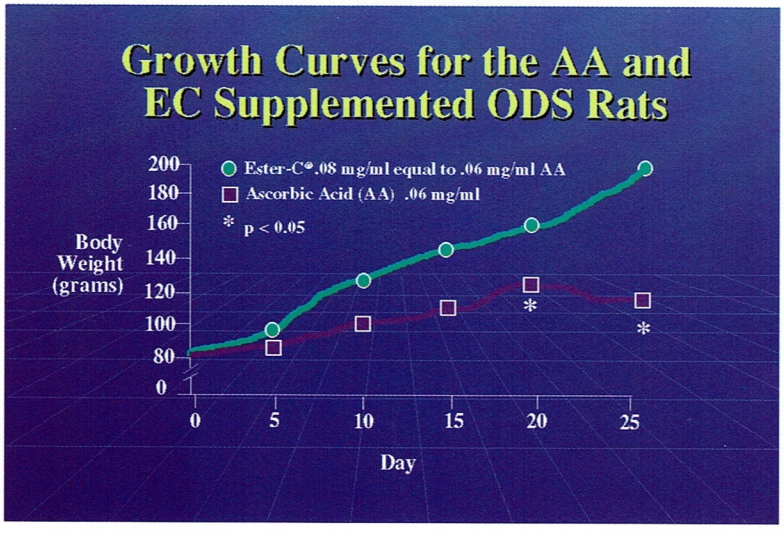
***Excerpts from Life Sciences, Vol. 48, pp.2275-2281, 1991***

The biological activity of the metabolites strongly differentiates **Ester-C®** ascorbate from conventional Vitamin C. This study clearly shows that, based on ascorbate activity equivalents, **Ester-C® is more potent and effective than ascorbic acid**.

The anti-scorbutic (anti-scurvy) potency of Ester-C®, a calcium ascorbate and calcium-threonate mixture, was compared with an ascorbic acid dose of equal ascorbate activity equivalents (AAE) for anti-scorbutic activity in the ODS rat....

Ester-C® rats had 3-4 times less, if any, scorbutic signs than ascorbic acid rats. The results clearly show that, based on ascorbate activity equivalents, Ester-C® has more available ascorbate activity / potency than ascorbic acid. This increased potency is believed to be due to the facilitated transport of AAE into the cell by the threonate (a normal *in vivo* metabolite of ascorbic acid) present in the Ester-C® product....

A normal metabolic product of L-ascorbic acid metabolism is calcium threonate. Bush and Verlangieri showed that Ester-C® administered at 10 mg/kg (AAE) versus ascorbic acid at AAE by gavage was absorbed more rapidly and excreted less rapidly in rats than ascorbic acid.... Maximum increase with calcium threonate was shown to be 177% greater than without calcium threonate....




The Ester-C® group had a significantly greater increase in body weight (125%) compared to the ascorbic acid group, which only had a 46% increase.... The difference in growth rate is most dramatic, with Ester-C® increasing body weight 3.5 times, a clear anti-scorbutic effect.... On the final day of the study, the ascorbic acid rats had 5 times more severe scorbutic signs than the Ester-C® rats.... It is clear from this study that, based on ascorbate activity equivalents, Ester-C® is 4-5 times more potent and effective than ascorbic acid in preventing scurvy. ... Since the transport of ascorbic acid has been shown to depend, in part, on insulin, and may involve an energy-dependent process, threonate may act in concert with insulin at the cellular receptor.... Regardless of the mechanism of the increased potency of ascorbate activity when delivered as Ester-C® as compared to ascorbic acid, the increase is significant. The presence of ascorbic metabolites enhances or facilitates the utilization of ascorbate activity in this scorbutic model.

Absorption curves favor **Ester-C®** mineral ascorbates over conventional Vitamin C or ascorbates. These research excerpts report that Ester-C® calcium ascorbate was absorbed **into the blood-stream faster** and **was held in the body longer** than ordinary Vitamin C (ascorbic acid).

# RELATIVE GASTRO-INTESTINAL ABSORPTION OF ESTER-C®

## An Acute Study on the Relative Gastro-Intestinal Absorption of A Novel Form of Calcium Ascorbate.

*By Marilyn J. Bush and A.J. Verlangieri*
Atherosclerosis Research Laboratories, Dept. of Pharmacology, School of Pharmacy, University of Mississippi

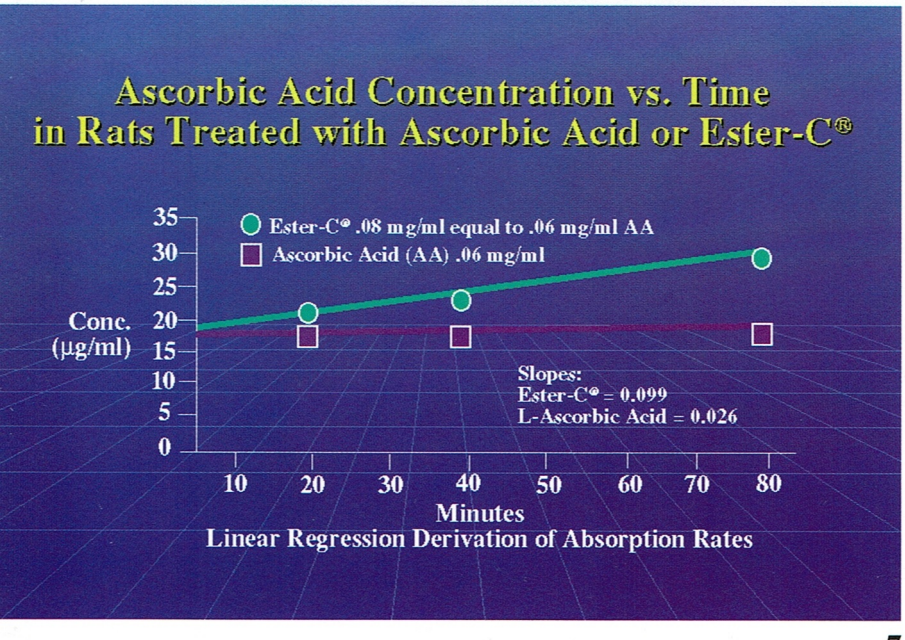
*Excerpts from Research Communications in Chemical Pathology and Pharmacology, Vol. 57, No. 1, July 1987*

Besides its established anti-scorbutic function, ascorbic acid (Vitamin C) is involved in many redox biosynthetic pathways, as in the formation of hydroxyproline and hydroxylysine during the synthesis of procollagen on the endoplasmic reticulum of connective tissue cells. Ascorbic acid may also serve in connective tissue metabolism as a carrier of activated sulfate needed for the synthesis of glycosaminoglycans (GAGs), as well as an inhibitor of aryl sulfatase B, which desulfates GAGs. Rapid cellular uptake of Vitamin C, along with delayed renal excretion, may promote optimal biochemical activity....

The results of this investigation suggest that **Ester-C®** is absorbed more readily than L-ascorbic acid, thus supporting the theory that it has a less ionic character, is more lipid soluble, and passes the mucosal barriers more rapidly than L-ascorbic acid....

Ascorbic acid was detected in the urine of L-ascorbic acid-treated animals twice as early as that of those treated with **Ester-C®**....

If the unique structure of **Ester-C®** promotes more rapid absorption and delayed excretion of ascorbic acid compared to the salt or acid forms of the vitamin, as the results of this experiment clearly imply, it may be advantageous in increasing circulating ascorbic acid concentration and promoting rapid tissue saturation.



# METABOLITES RESPONSIBLE FOR INCREASED ABSORPTION

## Acute Studies To Determine Relative Rates of Absorption of Ester-C® Calcium Ascorbate, U.S.P. Calcium Ascorbate, U.S.P. Calcium Ascorbate with an Added Metabolite, and "Another Commercial Ascorbate"

***By A.J. Verlangieri, Ph.D., F.A.C.N.***
Consulting Biochemical Pharmacologist and Toxicologist
Professor of Pharmacology and Toxicology, Department of Pharmacology,
School of Pharmacy, University of Mississippi

This study showed that **Ester-C®** is more **readily absorbed** than other ascorbates in raising and maintaining Vitamin C levels. This is crucial to the delivery and metabolic utilization of ascorbate to target tissues.

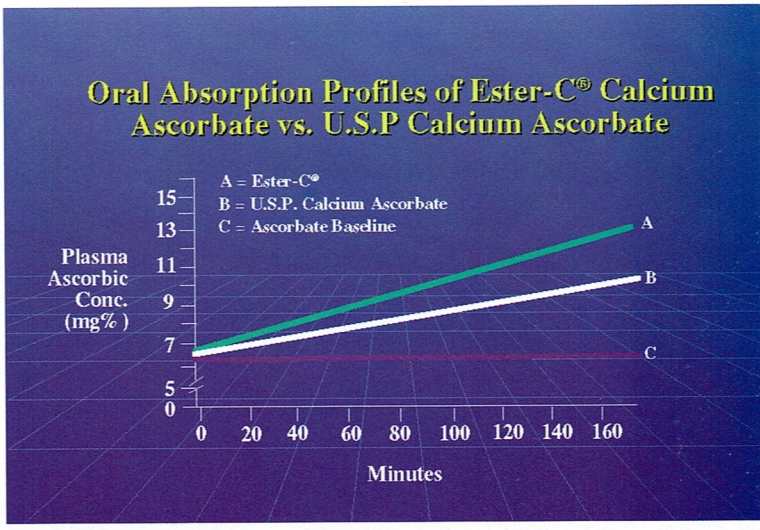
REVIEW

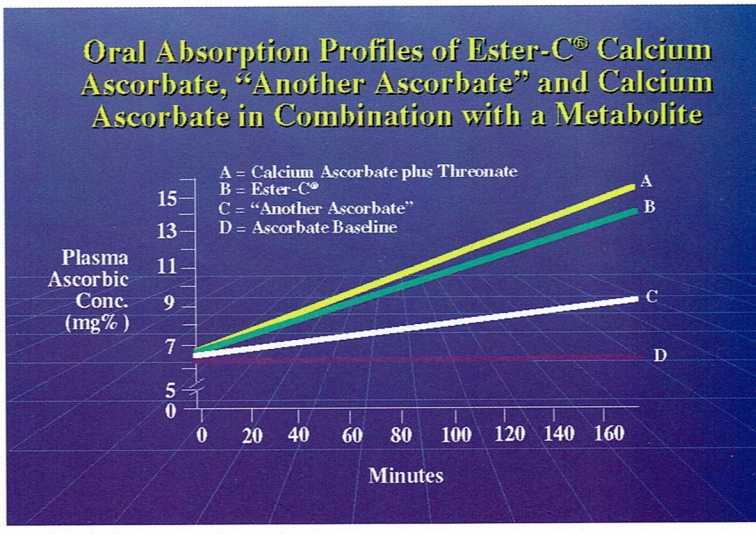
*In two related studies, groups of 5 male albino rats were fed different sources of ascorbate by stomach gavage in a single dose of 10 mg ascorbate equivalents. Blood samples were taken at 20, 40, 80, and 160 minutes after dosing. Ascorbate in the plasma fraction was analyzed by a high performance liquid chromatographic technique. Data at all timepoints were combined by statistical regression that best expressed the rate of ascorbate uptake into the plasma per minute.*

*Previous studies had shown that Ester-C® ascorbate is absorbed more readily than ascorbic acid. The first of these acute studies now shows that Ester-C® ascorbate is absorbed more rapidly than calcium ascorbate (0.04µg vs. 0.02µg of ascorbate per minute). The second of the two studies shows that both Ester-C® andU.S.P. calcium ascorbate plus a metabolite enter the blood compartment more rapidly than ascorbate derived from "another ascorbate" (food/C complex). In fact, the group receiving U.S.P. calcium ascorbate plus a metabolite achieved higher plasma levels faster than the other groups. There was a significant difference between the Ester-C® group and the metabolite group as compared to the group receiving "another ascorbate".*

*It is highly probable that the addition of a metabolite to U.S.P. calcium ascorbate is responsible for the increased absorption and attainment of higher ascorbate plasma levels. The presence of C metabolites suggests the mechanism by which Ester-C® reaches and maintains blood levels more rapidly.*

*It is apparent from the data that Ester-C® is more effective than "another ascorbate" in raising and maintaining ascorbate levels. This is a prerequisite to the delivery of ascorbate to target tissues where it interacts metabolically to achieve a therapeutic effect.*



White blood cells are known to have very high levels of Vitamin C, which they require for their metabolism. This in turn influences their ability to perform their important role as a **critical component** of the **immune system**.

High levels of intracellular ascorbate appear to aid the immune activity of white blood cells. **Ester-C®** ascorbate increased white blood cell ascorbate levels **more** than ascorbic acid. **Ester-C®** Vitamin C is ***The Power C***.

# A HUMAN BIOAVAILABILITY STUDY OF ESTER-C® ASCORBATE

## Comparative Studies of Ester-C® Versus L-Ascorbic Acid

***By Jonathan V. Wright, M.D., Raymond M. Suen, M.T. ASCP, & F. R. Kirk***

Meridian Valley Clinical Laboratory, Kent , Washington

***International Clinical Nutrition Review, Vol. 10, No. 1, Jan. 1990***

**REVIEW**

*The study was designed to compare the absorption, retention, and utilization of Ester-C® and ordinary ascorbic acid. Absorption was measured by Vitamin C levels in the plasma fraction of blood. Retention was inferred from urinary ascorbate levels, on the assumption that retention and utilization of ascorbate would limit the spillover into urine. And tissue utilization was assessed by measuring the amount of Vitamin C in the white blood cells, which are known to accumulate and utilize large amounts of Vitamin C for many physiological functions.*

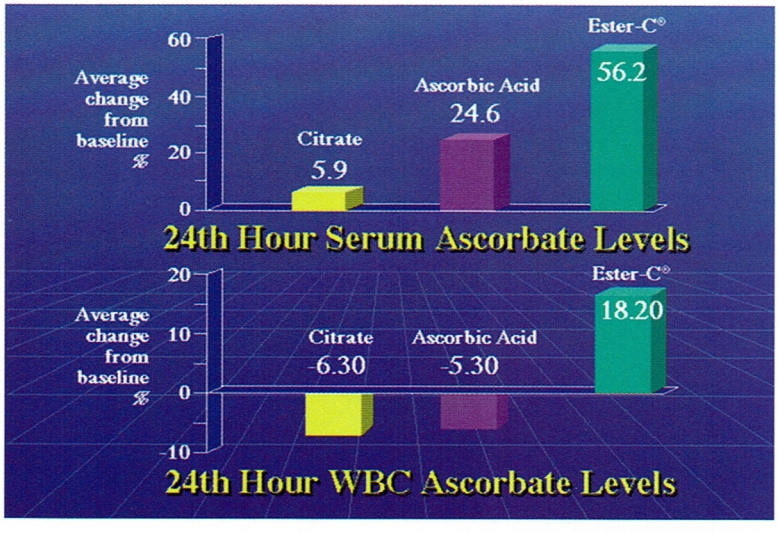
*Twelve men (aged 27-45) were placed on a low Vitamin C diet one week prior to experimentation. The men were initially divided into 3 groups of 4, and after an overnight fast each subject received a single oral dose of ascorbate (in product form) or of a placebo: Group 1 received ascorbic acid (3000 mg); Group 2 received Ester-C® calcium ascorbate (3000 mg of ascorbic acid equivalents); and Group 3 received 3000 mg of citric acid (placebo). Blood samples and 24-hr urine were collected prior to dosing. Blood samples were taken at 4, 8, and 12 hours after dosing for the determination of serum and white blood cell ascorbate levels. Another 24-hr urine collection was made after dosing in order to measure excreted ascorbate.*

**CONCLUSIONS**

Having compared L-Ascorbic Acid supplementation with Ester-C® supplementation, it appears that Ester-C® has several advantages:

1. Ester-C® produced higher intracellular ascorbate levels (as measured by White Blood Cells ascorbate) at all intervals measured.
2. 24-hour urinary ascorbate output was lower in the Ester-C® Group, confirming the above observation that more Vitamin C is retained when supplemented with Ester-C®.

Put simply, Ester-C® produced higher WBC levels and was excreted less in the urine than L-ascorbic acid.



# COMPARATIVE EFFICACY OF ESTER-C® ASCORBATE

*By Howard Hunt, Ph.D., and Thomas Rice, Ph.D.*
*The Life Management Group, La Jolla, CA*

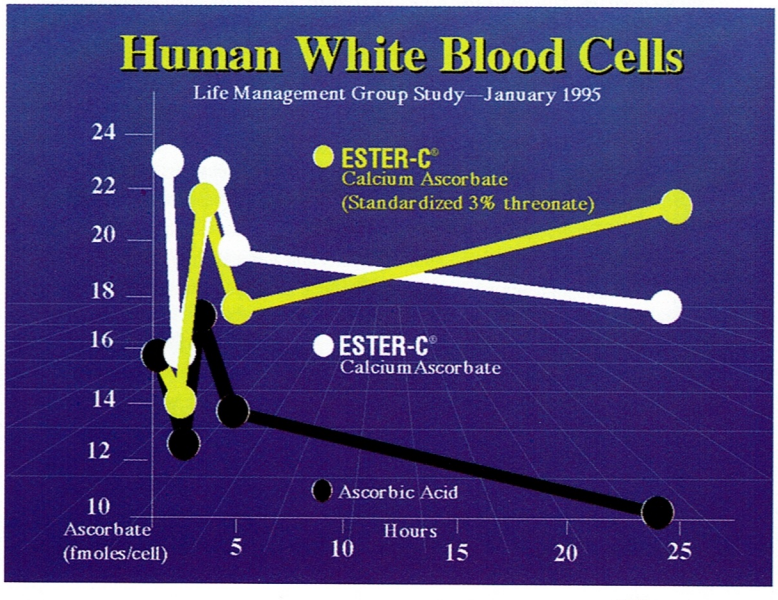
The cellular utilization of Vitamin C in Inter-Cal Ester-C® Calcium Ascorbate, with and without standardized 3% calcium threonate, was compared to pure ascorbic acid by measuring changes in white blood cells following a single oral dose of 1000 mg of Vitamin C (as ascorbic acid contained in product) in subject groups composed of 17-18 healthy men.

Subjects acclimatized to a low-Vitamin C diet for 12 days prior to supplementation. Blood samples were taken at 0 hr and again at 1, 2, 4, and 24 following ingestion of the test dose. Measurements of ascorbate concentration in white blood cells (buffy coat) were made by specific HPLC methods.

White cell ascorbate in the pure ascorbic acid group gradually declined by 24 hr but remained high in both the Ester-C® and the Ester-C® standardized with 3% threonate groups. At 24 hr, both Ester-C® groups showed white cell levels about twice as great as those in the pure ascorbic acid group. The Ester-C® product containing standardized threonate produced higher white cell ascorbate levels than the native Ester-C® product. Ascorbate levels at 24 hr in white cells of the Ester-C® calcium ascorbate group were significantly greater than those of the pure ascorbic acid group; this difference was highly statistically significant.

The study found a highly significant enhancement of cellular uptake of ascorbate from Ester-C® calcium ascorbate compared to pure ascorbic acid, as judged by white blood cell ascorbate levels.

The **Ester-C®** calcium ascorbate, with the naturally occuring C metabolite **threonate**, provides a superior way to build Vitamin C reserves in the important immuno-competent cells of the blood. **Additional levels** of threonate can enhance **absorption** and **efficacy** even further.



Researchers find that Vitamin C (ascorbic acid) is first oxidized to **DHA** before crossing the **cell membrane** and then is changed back to **ascorbic acid** once inside the cell. The carrier enzyme is the same enzyme that transports glucose (blood sugar) into the cell.

# MAXIMIZING INTRACELLULAR VITAMIN C LEVELS

## Mammalian Facilitative Hexose Transporters Mediate the Transport of Dehydroascorbic Acid

*By Juan Carlos Vera*, Coralla I. Rivas*, Jorge Fischbarg** and David W. Golde**

***Excerpts from Nature, Vol. 364, July 1, 1993, pp. 79-82***

* *Program in Molecular Pharmacology and Therapeutics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021*

** *Departments of Physiology and Cellular Biophysics and Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, NY 10032*

Although Vitamin C is critical to human physiology, it is not clear how it is taken up into cells. The kinetics of cell and tissue accumulation of ascorbic acid *in vitro* indicate that the process is mediated by specific transporters at the cell membrane....

In conclusion, our results with Xenopus oocytes and human neutrophils provide evidence indicating a direct role for the mammalian facilitative glucose transporters in the transport of ascorbic acid. Our data is consistent with the hypothesis that Vitamin C is transported by the glucose transporters as dehydroascorbic acid and then is rapidly reduced to ascorbic acid in the interior of the cell, a mechanism that allows for the accumulation of ascorbic acid against a concentration gradient.

---

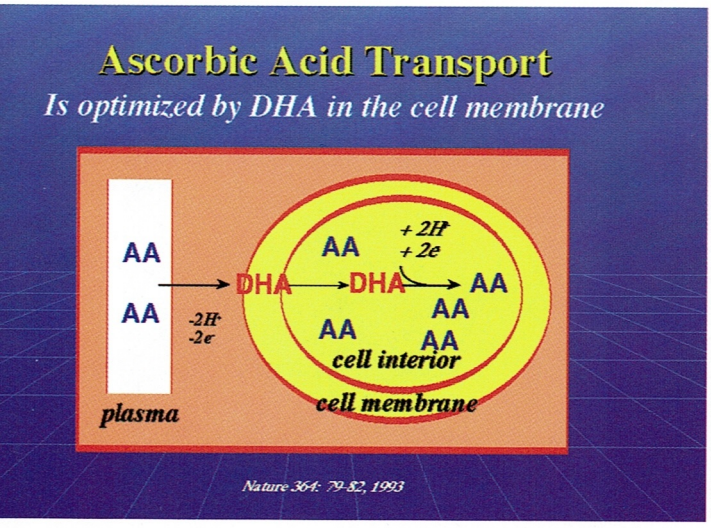
## Ascorbic Acid Recycling in Human Neutrophils

*By P.W Washko, Y. Wang, M. Levine*

***Excerpts from J. Biol. Chem. Vol. 268 , July 25,1993 pp. 15531-15535.***

*(Section of Cell Biology and Biochemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892)*

Ascorbic acid (Vitamin C) accumulation in activated human neutrophils is increased as much as 10-fold above the mM concentrations present in normal neutrophils. Internal concentrations as high as 14 mM are achieved when external vitamin is at physiologic concentration. The mechanism is by oxidation of external vitamin to dehydroascorbic acid, preferential transmembrane translocation of dehydroascorbic acid, and intracellular reduction to ascorbic acid within minutes. These data indicate that Vitamin C accumulation is enhanced in activated human neutrophils and that human neutrophils utilize and recycle oxidized external Vitamin C under physiologic conditions.



# ESTER-C®... THE SAFE VITAMIN C

## The Antimicrobial Capacity of Zinc Ascorbate

*By Steven J. Silverstein[1], D.M.D., M.P.H. and Charles I. Hoover[2], Ph. D.*

*[1]Professor, Department of Dental Public Health & Hygiene, University of California San Francisco School of Dentistry.*
*[2]Assistant Adj. Professor, Department of Stomatology, University of California San Francisco School of Dentistry.*

---

## Evaluation of the Tooth Erosive Characteristics of Ester-C® Zinc

*By Grayson Marshall, D.D.S., M.P.H., Ph.D. and Larry Watanabe*

*Department of Restorative Dentistry, University of California, San Francisco School of Dentistry*

---

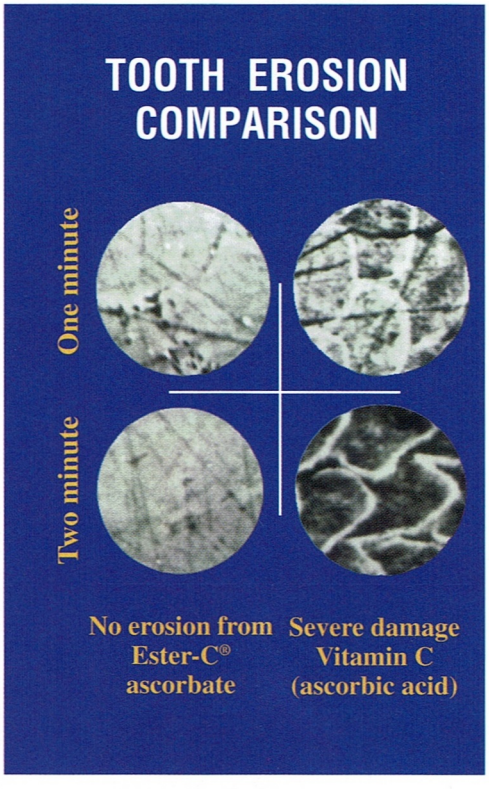
**REVIEW**

*Recently, much dental research has been focused on ways of reducing tooth decay by preventing the growth of bacteria that colonize the tooth surface. Two species in particular* –Streptococcus mutans *and* Streptococcus sobrinus–*are notorious culprits in plaque formation. It has become increasingly popular to add zinc compounds to mouth rinses and toothpastes for their bacteriostatic properties. It is not well known yet that one of the Ester-C® mineral ascorbates can accomplish the same result and provide necessary Vitamin C at the same time. Dr. Charles Hoover, working with Dr. Steven Silverstein at the University of California School of Dentistry in San Francisco, performed a study of the bacteriostatic effects of Ester-C® zinc ascorbate compared to a commonly used zinc salt. They grew Streptococcus bacteria in separate cultures so they could measure how many of the bacteria were able to attach themselves to a glass surface. Using this technique, they could mimic the way these bacteria create plaque on the surface of tooth enamel. They found that the Ester-C® zinc ascorbate was able to provide the same minimal inhibitory activity toward bacterial growth as an equivalent concentration of a zinc compound that has been commonly used in dental health products. In addition, the Ester-C® can provide important amounts of Vitamin C to strengthen periodontal tissues.*

*In another study, Dr. Marshall used a high-resolution microscope to produce photographs of the process of severe tooth erosion by ascorbic acid, the same source of Vitamin C that is in almost all chewable vitamins. He and his associate, Larry Watanabe, have documented this remarkably rapid process in a series of elegant experiments utilizing scanning electron microscopy. They showed that dilute solutions of plain ascorbic acid (containing amounts that would be typical in the mouth after chewing ascorbic acid supplements) caused definite erosion of intact human tooth enamal within one minute of exposure. After just a few minutes, the degree of enamel loss is readily apparent in the micrographs. By comparison, teeth exposed to equivalent concentrations of Vitamin C as the neutral Ester-C® calcium ascorbate remained completely unaffected.*

*Neutral Ester-C® ascorbate, which is neither acid nor alkaline, does not affect enamel in the least and should be the Vitamin C of choice for chewables and lozenges.*

Brief exposure to ordinary Vitamin C (ascorbic acid) can **erode** and **weaken** healthy tooth enamel. **Non-acidic Ester-C®** zinc ascorbate provides a **safe source** of Vitamin C to strengthen periodontal tissues and is an effective form of zinc that **inhibits** the bacteria associated with **tooth decay** and **plaque formation**.



Seventy eight percent (78%) of the dogs receiving **Ester-C®** calcium ascorbate at a dosage of 45 mg/kg body weight twice daily showed **improved mobility** within 4-5 days.

# VITAMIN C - NEW FORMS & NEW USES IN DOGS

*By L. Phillips Brown, D.V.M.*

***Proceedings of the American Holistic Veterinary Medical Association, pp.119-125, September 1994.***

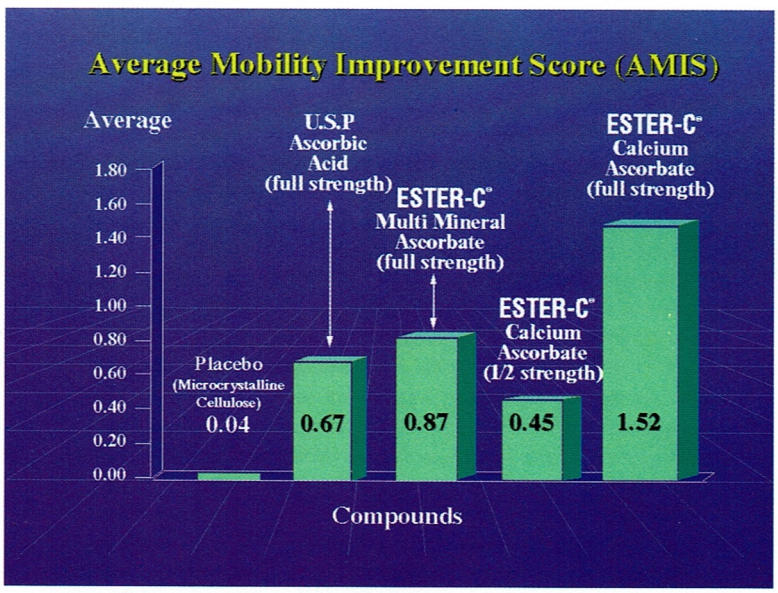
The effects of different forms of Vitamin C on various locomotor dysfunctions of dogs were investigated. Over one hundred case studies at an animal sanctuary were evaluated using varying strengths and combinations of mineral ascorbates, Vitamin C (ascorbic acid) and microcrystalline cellulose during a six month period. The results indicate that a patented Vitamin C ascorbate / Vitamin C metabolite complex administered orally may have application for the reduction of discomfort associated with chronic inflammatory disorders of the musculoskeletal system of dogs.

## CONCLUSION

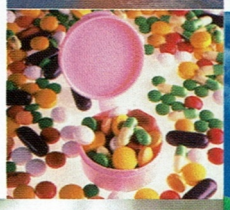
The consistency and degree of response demonstrates that Ester-C® calcium or mineral ascorbates provide symptomatic relief to dogs suffering from selected chronic joint and musculoskeletal disorders. The findings that the condition of many of the "improved" dogs deteriorated rapidly upon discontinuance of Ester-C® ascorbate further verifies its usefulness as primary or adjunctive therapy. These studies reinforce the earlier work of Docktor and Berge, which earlier demonstrated some positive effects of supplemental Ester-C® calcium ascorbate and its metabolites.

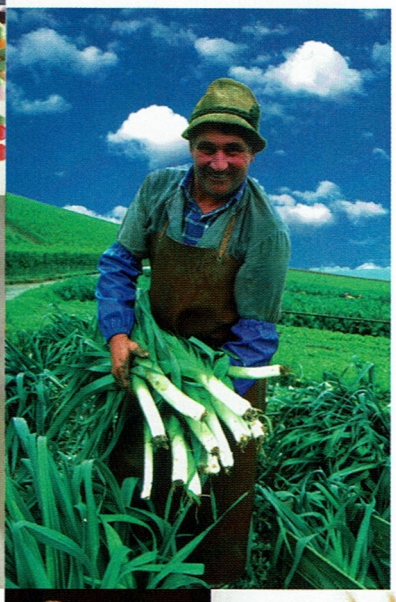
The study findings suggest that, although dogs can manufacture endogenous ascorbic acid, the amount produced in some dogs may not be sufficient to prevent or counteract stresses associated with aging, injury or joint malpositioning. Low or declining ascorbic acid availability within chrondrocytes may compromise collagen homeostasis, resulting in loss of tissue elasticity and/or compression resistance. Clinical signs of stiffness, discomfort and/or decreased mobility may be further exaggerated by age, exercise, inflammation or improper nutrition. A form of Vitamin C that would promote higher levels of intracelluar ascorbic acid without negative side effects, would be a useful alternative to steroids and aspirin.

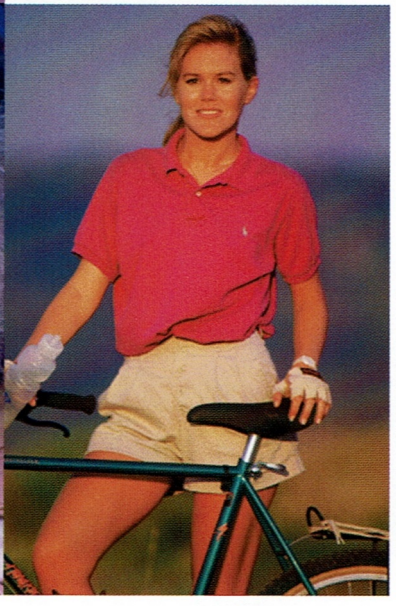
Response of degenerative joint disease in old dogs to supplementation with different ascorbate preparations. Responses of dogs during the entire study was quantitated as an Average Mobilty Improvement Score (AMIS) based on a 0-3 scale of functional improvement. Dogs fed the Ester-C® calcium ascorbate showed significantly better AMIS responses compared to ordinary ascorbic acid.



# FOUNDATIONS OF THE FUTURE







The future for **Ester-C®** ascorbates is bright. An ever-growing understanding of its metabolic mechanisms and antioxidant properties — as well as the unique and powerful roles its natural **metabolites** play — has opened a vista of potential new-product opportunities and applications research.

Promising early research and empirical observations have prompted aggressive scientific studies in the following disciplines:

- ***Food Technology***
- ***Cosmetic Science***
- ***Human Medicine***
- ***Dentistry***
- ***Veterinary Medicine***
- ***Agricultural Science***

**The Growing Family of Ester-C® Mineral Ascorbates**...Calcium, Magnesium, Zinc, Potassium, Sodium...

**For more information contact:**

## INTER-CAL CORPORATION

***Exclusive Manufacturers of Patented Ester-C® Vitamin C Products***


**World Headquarters:**
533 Madison Avenue
Prescott, Arizona 86301
U.S.A.
Telephone (520) 445-8063
Fax (520) 778-7986

**European Office:**
P.O. Box 2052
N-1522 Moss, Norway
Mobile Phone 47-90-02-43-86
Telephone 47-69-25-03-66
Fax 47-69-25-37-91



Ester-C® and the EC logo are licensed trademarks of the Inter-Cal Corporation
© Copyright 1995

*The Power C.*

# ESTER-C®

MINERAL ASCORBATES

- Patent Background
- Superior Absorption
- Cellular Transport
- New Understandings
- Foundations of the Future

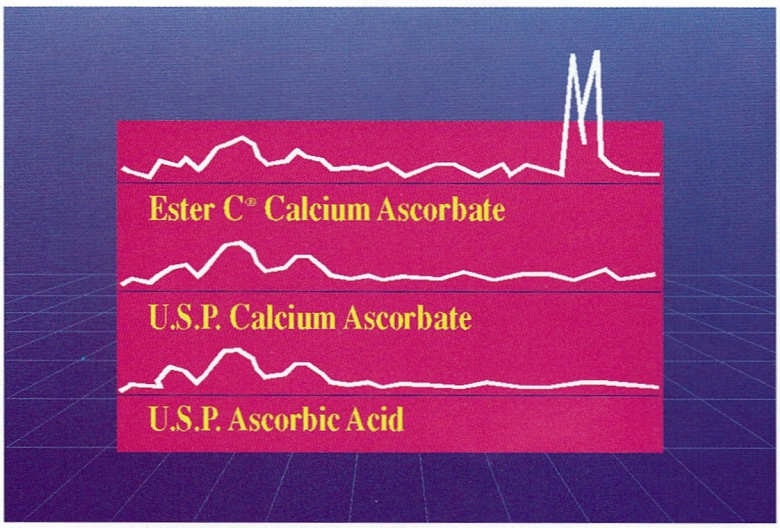
# RESEARCH SUMMARY

The need for vitamin C has never been greater. Stress, environmental pollution, age and health, as well as biochemical individuality, all help to dictate our daily requirements for this essential nutrient. Unlike most other animals, humans cannot make their own vitamin C. With vitamin C being part of more than 300 bodily functions, life itself depends on daily replenishment. Is there a best form of this vital nutrient?

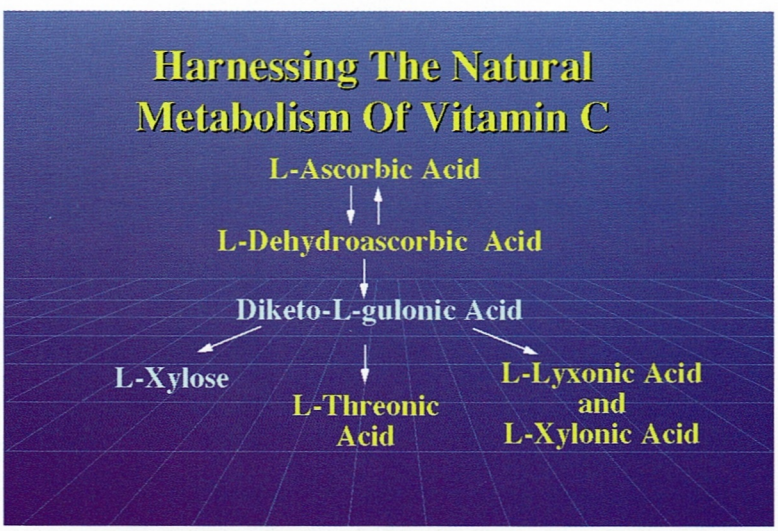
**Ester-C®** ascorbate, a revolutionary form of **vitamin C**, is the most bioavailable form developed. **METABOLITES** in Ester-C® ascorbate help the vitamin get into the bloodstream faster and in larger amounts, penetrate white blood cells more efficiently, and waste only a fraction of what other vitamin Cs "send down the drain."

Conventional mineral ascorbates are manufactured using either alcohol or acetone. During our exclusive **water-based manufacturing process**, specifically controlled amounts of the vitamin C in the Ester-C® ascorbate undergo **structural changes similiar to those that happen in the body** to produce bioavailable metabolites.

# THE KEY TO THE POWER OF ESTER-C® BRAND — *METABOLITES.*




*These three graphs are from actual identical segments of Nuclear Magnetic Resonance (NMR) spectra of U.S.P. ascorbic acid, U.S.P. calcium ascorbate and* ***Ester-C® Calcium Ascorbate****. An NMR is a "fingerprint" of the chemical structure of a compound, and the height of the peaks at specific points indicates the amount of a certain material present in a mixture. These graphs are very similiar until the point at the far right is reached. This area indicates the amount of the natural C metabolite, threonate, in each form of vitamin C.*




*The key components of patented Ester-C® Mineral Ascorbates.*

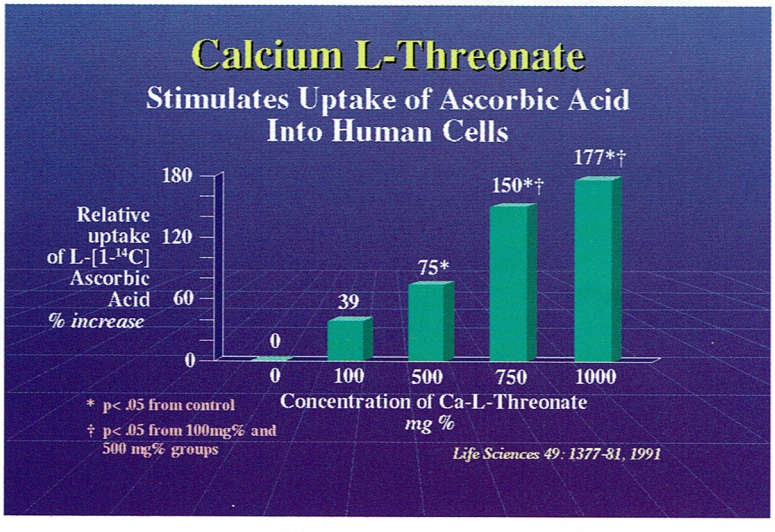
# THREONATE ACCELERATES VITAMIN C UPTAKE

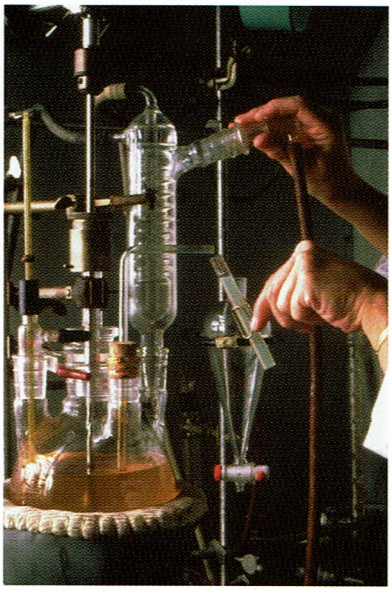
## Stimulatory Action of Calcium L-Threonate on Ascorbic Acid Uptake by a Human T-Lymphoma Cell Line

***By M.J. Fay and A.J. Verlangieri***
Department of Pharmacology, School of Pharmacy, University of Mississippi

*Life Sciences, Vol. 49, August 1991, pp.1377-1381.*
*The following abstract was presented at a meeting of the Federation of American Societies for Experimental Biology (April 21-25, 1991).*

The metabolism of ascorbic acid (AA) involves the oxidation of AA to dehydroascorbic acid which subsequently undergoes a hydrolytic ring cleavage to form 2, 3-diketo-1-gulonic acid. Diketo-1-gulonic acid may become decarboxylated forming L-lyxonic acid, L-xylose, and L-xylonic acid, or it may be further oxidized to form threonic and oxalic acids. Although much research has been done concerning the biological and pharmacological effects of AA, very little work has been done with the AA metabolites. In the present studies we investigated the effects of preincubation (1 hour) of human T-lymphoma cells with increasing concentrations of calcium L-threonate (0-1,000 mg %) on the uptake (30 min.) of L-[14 C}AA (1.25 mg % unlabeled / 0.125uCi labeled). The results indicate that calcium L-threonate significantly increases cellular AA uptake in a dose dependent manner, with the highest level of threonate increasing uptake approximately 177% above control levels. These results correlate with *in vivo* studies showing that Ester-C® (a calcium ascorbate supplement containing AA metabolites) is absorbed more rapidly than AA. The facilitation of cellular AA uptake and subsequent increase in cellular free radical scavenging potential may have important clinical therapeutic implications.

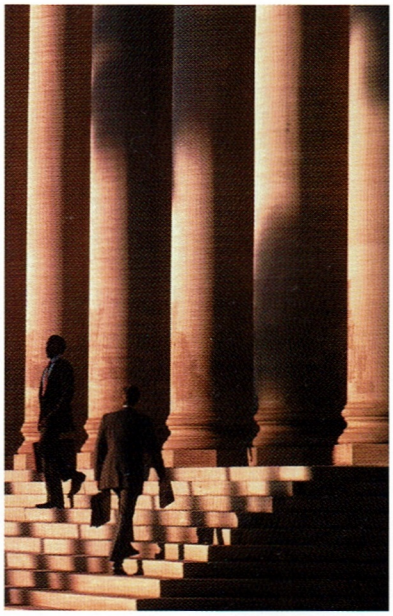


As the graph on the opposite page indicates, Ester-C® calcium ascorbate contains a significant level of the natural C metabolite **THREONATE**, compared with other vitamin C supplements.

**METABOLITES** are the key to enhancing blood levels and tissue concentration of vitamin C. **THREONATE**, which is the dominant metabolite in the Ester-C® complex, is body-ready for these roles in each of the various Ester-C® mineral ascorbates.

# THE SUPERIORITY OF ESTER-C®'S UNIQUE NUTRIENT FORM



Based on the strength of the research confirming that **metabolites enhance bioavailability and absorption** of vitamin C, on April 18, 1989, the United States Department of Commerce Patent and Trademark Office issued patent No. 4,822,816, which recognizes the superiority of this unique product.

**United States Patent** [19]
Markham

[11] Patent Number: 4,822,816
[45] Date of Patent: Apr. 18, 1989

[54] COMPOSITIONS AND METHODS FOR ADMINISTERING VITAMIN C

[75] Inventor: Richard G. Markham, Prescott, Ariz.

[73] Assignee: Oxycal Laboratories, Inc., Prescott, Ariz.

[21] Appl. No.: 36,598

[22] Filed: Apr. 10, 1987

[51] Int. Cl.[4] .....A01N 43/08; A61K 31/335

[52] U.S. Cl. .....514/474

[58] Field of Search.....514/474, 922, 163, 460, 514/473; 424/10

[56] References Cited
U.S. PATENT DOCUMENTS

3,459,863 8/1969 Apelian et al. .....514/474
3,781,423 12/1973 Aoka et al. .....514/474

OTHER PUBLICATIONS

Lewin-Vitamin C its Molecular Biology and Medical Potential, Academic Press (1976)

*Primary Examiner*- Douglas W. Robinson
*Assistant Examiner*- Roger E. Gobrogge
*Attorney, Agent, or Firm*- William H. Drummond

[57] ABSTRACT

A composition useful for administering Vitamin C to a subject comprises a compound having Vitamin C activity and at least one compound selected from the class consisting of the aldono-lactones and edible salts of L-threonic, L-xylonic and L-lyxonic acids.

High Vitamin C levels in the human body are established by orally administering to a human subject an effective amount of this composition.

2 Claims, No Drawings

PATENT CLAIMS ALLOWED

1. A vitamin composition comprising:
(a) an effective amount of a compound having Vitamin C activity, selected from the group consisting of (i) dehydroascorbic acid, (ii) L-ascorbic acid and the edible salts thereof, and

(b) at least one compound selected from the group consisting of the aldono-lactones of L-threonic acid, L-xylonic acid, L-lyxonic acid and the edible salts of L-threonic acid, L-xylonic acid and L-lyxonic acids, in an amount effective to increase the human body absorption rate of said Vitamin C compound.

A method for establishing high Vitamin C levels in the human body which comprise the step of orally administering to a subject an effective amount of the composition of claim 1.

The United States of America
The Commissioner of Patents and Trademarks
Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.
Therefore, this
United States Patent
Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

# RELATIVE GASTRO-INTESTINAL ABSORPTION OF ESTER-C®

## An Acute Study on the Relative Gastro-Intestinal Absorption of A Novel Form of Calcium Ascorbate.

***By Marilyn J. Bush and A.J. Verlangieri***
Atherosclerosis Research Laboratories, Dept. of Pharmacology, School of Pharmacy, University of Mississippi

*Excerpts from Research Communications in Chemical Pathology and Pharmacology, Vol. 57, No. 1, July 1987*

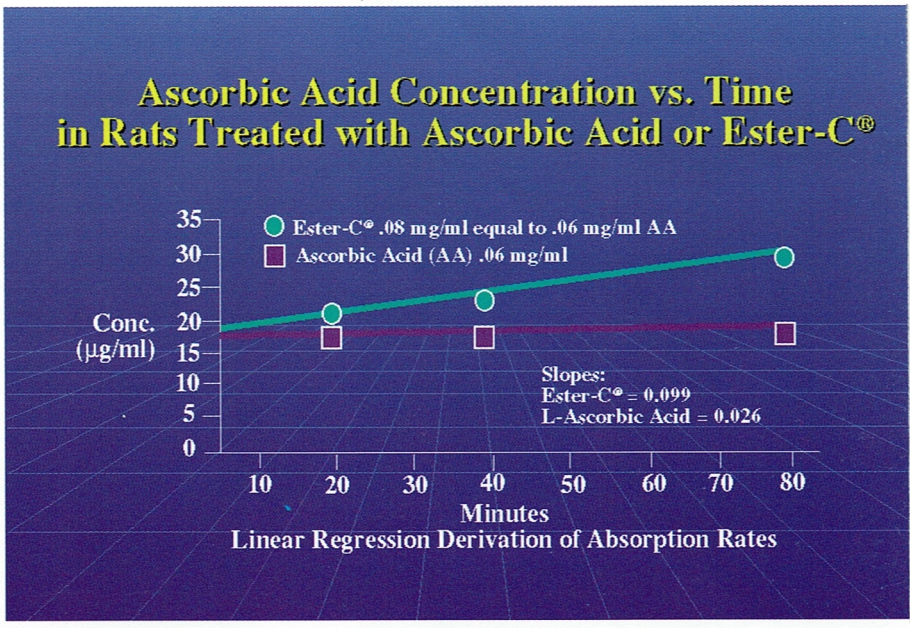
Besides its established anti-scorbutic function, ascorbic acid (vitamin C) is involved in many redox biosynthetic pathways, as in the formation of hydroxyproline and hydroxylysine during the synthesis of procollagen on the endoplasmic reticulum of connective tissue cells. Ascorbic acid may also serve in connective tissue metabolism as a carrier of activated sulfate needed for the synthesis of glycosaminoglycans (GAGs), as well as an inhibitor of aryl sulfatase B, which desulfates GAGs. Rapid cellular uptake of vitamin C, along with delayed renal excretion, may promote optimal biochemical activity....

The results of this investigation suggest that **Ester-C®** is absorbed more readily than L-ascorbic acid, thus supporting the theory that it has a less ionic character, is more lipid soluble, and passes the mucosal barriers more rapidly than L-ascorbic acid....

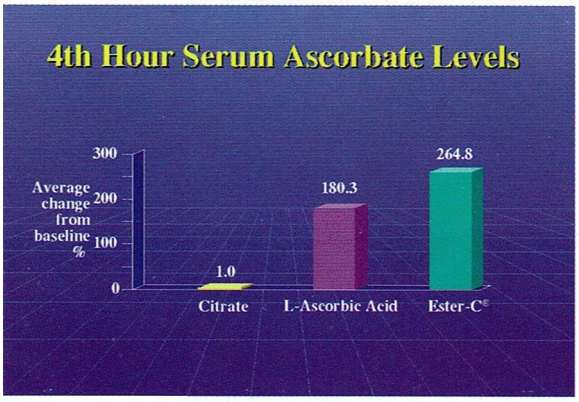
Ascorbic acid was detected in the urine of L-ascorbic acid-treated animals twice as early as that of those treated with **Ester-C®**.

If the unique structure of **Ester-C®** promotes more rapid absorption and delayed excretion of ascorbic acid compared to the salt or acid forms of the vitamin, as the results of this experiment clearly imply, it may be advantageous in increasing circulating ascorbic acid concentration and promoting rapid tissue saturation.

Absorption curves favor Ester-C® mineral ascorbates over conventional vitamin C or ascorbates. The adjacent research excerpts report that Ester-C® calcium ascorbate was absorbed **into the bloodstream twice as fast** and **was held in the body twice as long** as ordinary vitamin C (ascorbic acid).



White blood cells are known to have very high levels of vitamin C, which they require for their metabolism. This in turn influences their ability to perform their important role as a **critical component of the immune system**.




High levels of intracellular ascorbate appear to assist the immune activity of white blood cells. **Ester-C®** ascorbate increased white blood cell ascorbate levels up to **4 times more** than ascorbic acid, with as little as **one third as much wasted.**

# A HUMAN BIOAVAILABILITY STUDY OF ESTER-C® ASCORBATE

## Comparative Studies of Ester-C® Versus L-Ascorbic Acid

***By Jonathan V. Wright, M.D., and Raymond M. Suen, M.T. ASCP***

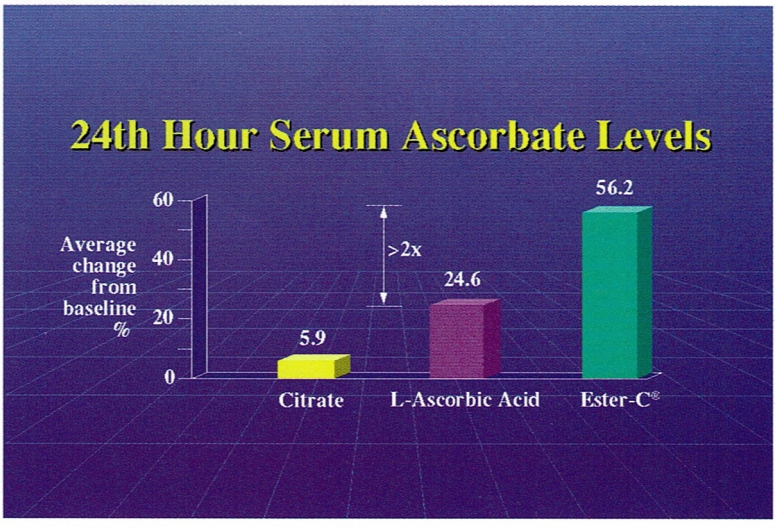
Meridian Valley Clinical Laboratory, Kent , Washington, Feb. 28, 1987

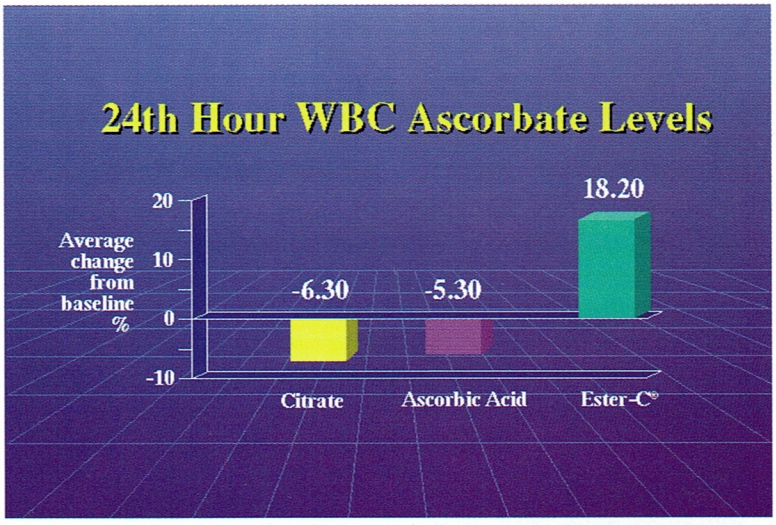
*Excerpts from International Clinical Nutrition Review, Vol. 10, No. 1, Jan. 1990*

### Summary of Protocol

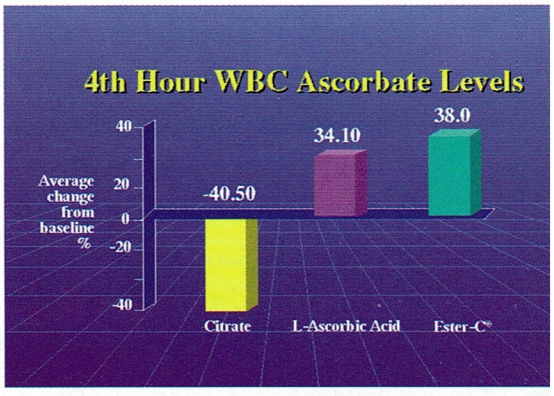
1. 12 men, ages 27 to 45, were studied
2. All were instructed that they should be on a low vitamin C diet for one week prior to the study. No citrus products, and no large amounts of green leafy vegetables.
3. Following overnight fast, blood and urine samples were taken. White blood cell (WBC) and 24-hour urinary ascorbate and oxalate levels were determined and correlated with serum ascorbate levels.
4. The 12 men were divided into three groups and were asked to take the following supplements:
   A. Ester-C® Group
   B. Ascorbate Group
   C. Citric Acid Group
5. All 12 men were asked to continue on the low vitamin C diet. Blood samples were taken at 0, 4, 8, and 24 hours after morning ingestion of the designated supplements. Urinary 24-hour ascorbates and oxalate levels were determined. After a washout period (varying from two days to several days, due to job situation of the participants) the groups were asked to switch to another supplement, as follows:
   A. Ester-C® Group to Citric Group
   B. Citrate Group to Ascorbic Acid Group
   C. Ascorbate Group to Ester-C® Group
6. Supplements were taken at the same level (the equivalent of 3,000 mg of assayable vitamin C for groups A and B and 3,000 mg of Citric Acid for Group C) by all three groups. Blood samples were again drawn at 0, 4, 8, and 24 hours from the time of ingestion. A 24-hour urine sample was also collected by all 12 participants.

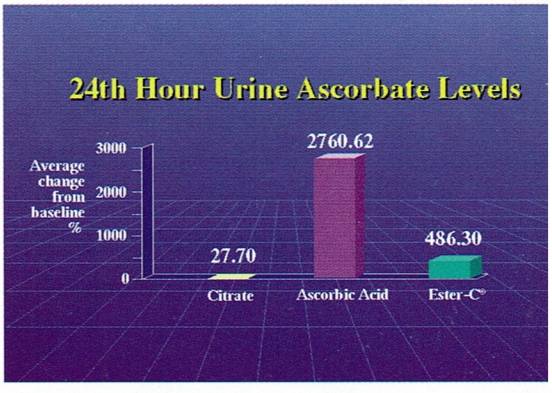
Serum ascorbate, white blood cell ascorbate and 24-hour urinary ascorbate output as well as oxalate were analyzed.

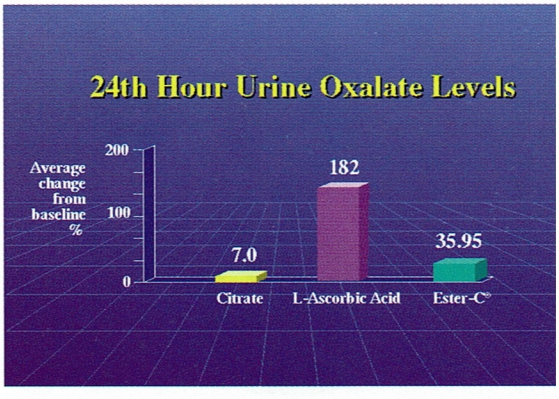





Faster absorption into the bloodstream and white blood cells, maintaining higher levels for longer periods, low oxalate production, and reduced waste all show that **Ester-C®** vitamin C is ***The Power C.***




## Discussion

**Serum Ascorbate Level:** At 4, 8 and 24 hours later, Ester-C® Group tends to have a higher serum ascorbate level as compared to both the Citrate Group and the L-Ascorbic Acid Group.

**WBC Ascorbate Level:** Although all 8th hour WBC Ascorbate Groups showed an average decrease, Ester-C® had the smallest percentage decrease. 4- and 24-hour measurement showed that Ester-C® was able to maintain the highest white blood cell ascorbate level.

**24-Hour Urine Ascorbate Output:** In average percentage change, the Ester-C® Group had less ascorbate output than the L-Ascorbic Acid Group.

**24-Hour Urinary Oxalate Output:** Oxalate output is greatly decreased in the Ester-C® Group as compared to the L-Ascorbic Acid Group. This would mean that while taking Ester-C® as a supplement, a person has less chance of forming oxalate-containing kidney calculi than a person taking L-Ascorbic Acid.

## Conclusion

Having compared L-Ascorbic Acid supplementation with Ester-C® supplementation, it appears that Ester-C® has several advantages:

1. Ester-C® produced higher intracellular ascorbate levels (as measured by WBC ascorbate) at all intervals measured.
2. 24-hour urinary ascorbate output was lower in the Ester-C® Group, confirming the above observation that more vitamin C is retained when supplemented with Ester-C®.
3. Ester-C® is associated with less urinary oxalate excretion, presumably creating less risk of oxalate kidney stones.

Put simply, Ester-C® produced higher WBC levels, was excreted less in the urine and was associated with lower urinary oxalate output than L-Ascorbic Acid.

### A Human Clinical Study of Ester-C® vs. L-Ascorbic Acid 24-Hour Results

| | CITRATE | ASCORBATE | ESTER-C® | ESTER-C HAS |
|---|---|---|---|---|
| Serum (Blood) | + 05% | + 25% | + 55% | 2 X more in blood |
| WBC (Tissues) | - 06% | - 05% | + 18% | 4 X more in tissues |
| Excretion | 0 | -2600% | -800% | 3 X less excreted |
| Oxalates | + 05% | + 160% | + 30% | 5 X less oxalates |

Many people experience **Acid Rejection Syndrome** when acidic vitamin C enters the more alkaline environment of the lower digestive tract. Common side effects are flatulence, diarrhea and discomfort.

Modern science tried several ways to reduce acid stress. So called "buffered" vitamin C, traditional mineral ascorbates, timed-release and "natural" vitamin C supplements offered reduced adverse side effects, but their bioavailability was still limited.

Ester-C® vitamin C, with a **neutral pH**, is naturally processed in purified water, not solvents, and is proven to be up to **4 times more bioavailable** than ordinary vitamin C.

# INCREASED ABSORPTION AND DECREASED EXCRETION

## An Acute Study To Determine The Relative Rate of Absorption and Excretion of U.S.P. Calcium Ascorbate and Ester-C® Calcium Ascorbate

***By A.J. Verlangieri, Ph. D., F.A.C.N.***
Consulting Biochemical Pharmacologist and Toxicologist
Professor of Pharmacology and Toxicology, Department of Pharmacology, School of Pharmacy, University of Mississippi

Ten Charles-River® Sprague-Dawley albino rats (384-411 gms, males) were acclimated for seven days prior to beginning the study....

The rats were then randomly assigned to two groups (5/group). Group 1 rats received 10 mg/kg U.S.P Calcium Ascorbate by gavage (1 ml., in deionized water). Group 2 rats received Ester-C® dose corrected to 10 mg/kg ascorbate and administered as in Group 1.

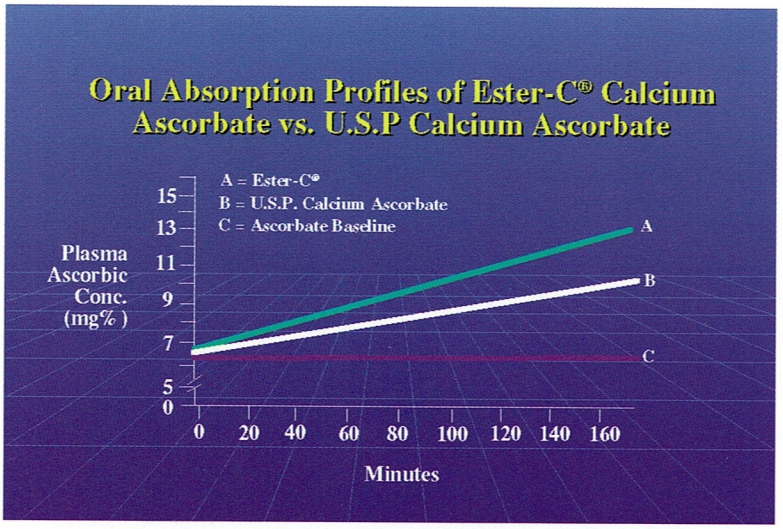
After each animal had been dosed, they were placed in Nalgene® metabolic cages. Urine was anlyzed as it was produced for ascorbic acid. (Uralyte Fast Tabs, 0,+,++)....

Blood samples were taken at 20, 40, 80 and 160 minutes after dosing. The blood was fractionated into plasma for ascorbate analysis. (HPLC method as previously described, J. Liquid Chromotography, 6:7, 1319-1332, 1983)....

The figure below is the regression fit for the plasma data. Slopes or rate of uptake are expressed in µgs ascorbate appearing in the plasma per minute. This rate is equivalent to µgs ascorbate absorbed per minute. The experimental animals urinated very little during the study. The number of data points for ascorbate analysis are too scant to comment on....

As studies have previously shown (Research Communications in Chemical Pathology, 57(1) 137-140,1988), Ester-C® is absorbed more readily than ascorbic acid. This study now shows Ester-C® to be absorbed more rapidly than Calcium Ascorbate (.04 µgs/min. vs. .02 µgs.min.). While the urine data are too scant to analyze, this rate difference is most likely due to a combination of both increased absorption and reduced excretion....

The structure of Ester-C® is apparently unique and differs from U.S.P. Calcium Ascorbate. Increased absorption of ascorbate with decreased excretion is advantageous in increasing circulation ascorbate levels and tissue levels. Such effects are paramount to effective therapeutic uses of ascorbate.



# METABOLITES RESPONSIBLE FOR INCREASED ABSORPTION

## An Acute Study To Determine The Relative Rate of Absorption and Excretion of Ester-C® Calcium Ascorbate, U.S.P. Calcium Ascorbate with an Added Metabolite and "Another Commercial Ascorbate."

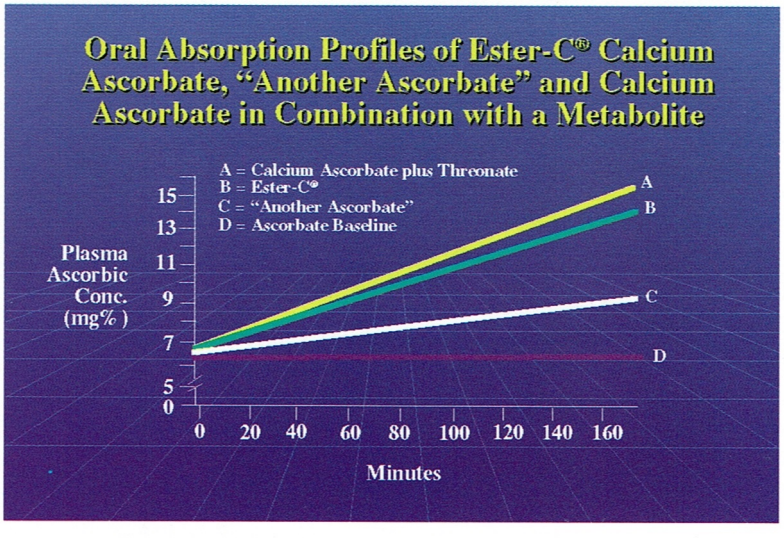
***By A.J. Verlangieri, Ph. D., F.A.C.N.***
Consulting Biochemical Pharmacologist and Toxicologist
Professor of Pharmacology and Toxicology, Department of Pharmacology, School of Pharmacy, University of Mississippi

The figure below shows that both Ester-C® and U.S.P. Calcium Ascorbate with a Metabolite enter the bloodstream compartment more rapidly than ascorbate derived from "another ascorbate". In fact, the U.S. P. Calcium Ascorbate plus a Metabolite group achieved higher plasma levels faster than the other two groups. There is a significant difference between the Ester-C® group and the Metabolite group as compared to "another ascorbate" group. The animals did not produce adequate urine at the sample times to analyze urine ascorbate. However, the Metabolite group had the least positive urine at 3 hours. This suggests less excretion over the experimental period....

It is highly probable that the addition of a Metabolite to U.S.P. Calcium Ascorbate is responsible for the increased absorption and attainment of higher ascorbate plasma levels. It has been found that Ester-C® contains significant levels of Metabolites. This clarifies the results of another study comparing U.S.P Calcium Ascorbate and Ester-C®. Ester-C® had a more rapid absorption than U.S.P Calcium Ascorbate and is most probably due to the presence of these Metabolites....

The addition of a Metabolite increases the uptake of U.S.P. Calcium Ascorbate and indicates the mechanism by which Ester-C® reaches and maintains blood levels more rapidly....

It is apparent from the data that Ester-C® is more effective than "another ascorbate" in raising and maintaining ascorbate levels. This is a prerequisite to the delivery of ascorbate to target tissues where it interacts metabolically to achieve a therapeutic effect.




This study showed that "Ester-C® is more effective than 'another (commercially available) ascorbate' in raising and maintaining ascorbate levels." This is crucial to the delivery and metabolic utilization of ascorbate to target tissues.

Researchers find that vitamin C (ascorbic acid) is first oxidized to **dehydroascorbic acid (DHA) before crossing the cell membrane** and then is **changed back to ascorbic acid** once inside the cell. The carrier enzyme(s) is the same enzyme(s) that transports glucose (blood sugar) into the cell.

# MAXIMIZING INTRACELLULAR VITAMIN C LEVELS

## Mammalian Facilitative Hexose Transporters Mediate the Transport of Dehydroascorbic Acid

*By Juan Carlos Vera*, Coralla I. Rivas*, Jorge Fischbarg** and David W. Golde**

*Excerpts from Nature, Vol. 364, July 1, 1993, pp. 79-82*

* *Program in Molecular Pharmacology and Therapeutics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021*

** *Departments of Physiology and Cellular Biophysics and Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, NY 10032*

Although vitamin C is critical to human physiology, it is not clear how it is taken up into cells. The kinetics of cell and tissue accumulation of ascorbic acid *in vitro* indicate that the process is mediated by specific transporters at the cell membrane....

We use the *Xenopus laevis* oocyte expression system to show that the mammalian facilitative hexose transporters are efficient transporters of the oxidized form of vitamin C (dehydro-ascorbic acid). Two transport pathways, one with low affinity and one with high affinity for dehydroascorbic acid, were found in oocytes expressing the mammalian transporters, and these oocytes accumulated vitamin C against a concentration gradient when supplied with dehydroascorbic acid. We obtained similiar results in experiments using normal human neutrophils. These observations indicate that mammalian facilitative hexose transporters are a physiologically significant pathway for the uptake and accumulation of vitamin C by cells, and suggest a mechanism for the accumulation of ascorbic acid against a concentration gradient....

The observation that some cells accumulate high concentrations of ascorbic acid against a concentration gradient has been used as an argument against the participation of facilitative glucose transporters in the cellular uptake of ascorbic acid. We found that oocytes expressing glut-1 accumulated about 2.5 times more radioactive vitamin than the amount expected from simple uptake into a cell with an average internal volume of about 0.4µl (for a full-grown stage-6 oocyte). This effect was dependent on the concentration of dehydro-ascorbic acid during the incubation, and was most noticeable in the low micromolar concentration range....Thus, ascorbic acid can be accumulated against a concentration gradient by a mechanism involving facilitated uptake of the oxidized form and retention of the reduced ascorbic acid.

We also analyzed the transport of ascorbic acid by human neutrophils, and the results closely matched those from *Xenopus* oocytes expressing mammalian glucose transporters. Under conditions that prevented spontaneous oxidation of ascorbic acid, the neutrophils did not take up the reduced form of ascorbic acid, but rapidly transported dehydroascorbic acid. Neutrophils accumulated ascorbic acid against a concentration gradient when exposed to dehydroascorbic acid, and two transport activities were detectable with different affinities for dehydroascorbic acid.... Finally, we documented competitive inhibition of the transport of ascorbic acid by glucose with a $K_i$ of 9 mM. These observations strongly support the concept that the glucose transporters are a physiologically significant pathway for the uptake of ascorbate in human cells.

In conclusion, our results with *Xenopus* oocytes and human neutrophils provide evidence indicating a direct role for the mammalian facilitative glucose transporters in the transport of ascorbic acid. Our data is consistent with the hypothesis that vitamin C is transported by the glucose transporters as dehydroascorbic acid and then is rapidly reduced to ascorbic acid in the interior of the cell, a mechanism that allows for the accumulation of ascorbic acid against a concentration gradient.

# ESTER-C® COMPLEX MIRRORS NATURE

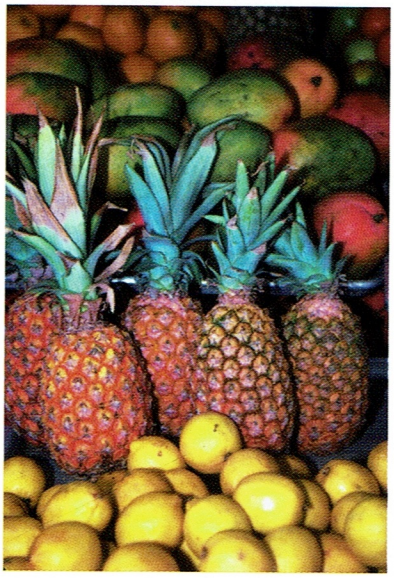
*By A.J. Verlangieri, Ph.D., F.A.C.N*
Professor of Toxicology and Pharmacology
Director of the Atherosclerosis Research Laboratories
University of Mississippi, University, MS 38677

I have carefully reviewed the literature concerning adverse effects of ascorbic acid (AA) and dehydroascorbic acid (DHA) since 1986. This search revealed no significant citations of adverse effects of DHA in animals or humans. In fact, it is stressed that the DHA/AA ratio is most important in considering the action of ascorbate. DHA and AA are a redox couple, meaning they work together to carry out redox reactions in normal metabolism. In addition, DHA is the preferred form of the vitamin for cellular transport. At the cell membrane, AA is converted to DHA by ascorbate oxidase: NAD (P)$^+$. Once inside the cell, DHA is converted back to ascorbate by dehydroascorbic acid reductase: NAD (P)+:GSH. As one can see, DHA is a very important partner in ascorbate biological action.

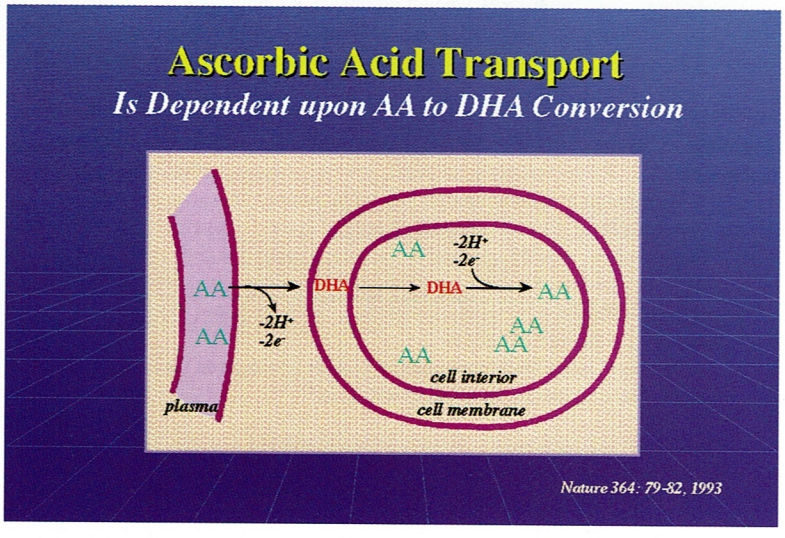
As mentioned, the DHA/AA ratio is very critical. The following ratios are found: Liver 0.1, Adrenals 0.1, and Heart 0.8. The high redox activity and energy requirements of the tissue, such as heart, is reflected by higher DHA/AA ratios. Supplements should contain or provide the same ratio of DHA/AA as found in tissues to maintain the redox balance or, at the very least, should not exceed this normal tissue ratio.

Ester-C® has a DHA/AA of 0.1, the same ratio found in the liver and the adrenals and yet less than the ratio found in heart muscle. This is ideal, since this supplement will not pertubate the normal ratios found in these tissues. In addition, plasma has a normal DHA/AA ratio of 0.5. Therefore, ingestion of Ester-C® will not affect this ratio either and not disturb the redox couple and metabolic redox reactions. This could imply that there is a disadvantage to taking L-Ascorbic acid as a supplement without DHA present.

It is interesting to note that several foods, i.e. broccoli, carrots, celery and cucumber, have DHA/AA ratios ranging from 2 to 0.22. Orange juice has a ratio of 0.1 and infant formula, 0.2. All of the above have the potential to pertubate the normal body DHA/AA ratios, while Ester-C® does not.



Dehydroascorbic acid is the preferred form of vitamin C for cellular transport. The **Ester-C® ascorbates have a DHA/AA ratio of 0.1, the same ratio found in the liver and the adrenals.**



Research identifies a unique electro-chemical potential between oxidized (DHA) and reduced (ascorbic acid) forms of vitamin C that **may optimize growth,** along with other vital biological functions.

# ASCORBATE IN THE GROWTH PROCESS

## The Role of Ascorbate in Biomembrane Energetics

*By D. James Morre*, Frederick L. Crane**, Iris L. Sun**, & Placido Navas*, ***
*From The Department of Medicinal Chemistry, ** Department of Biological Sciences, Purdue University, West Lafayette, IN 47907
*Excerpts from Third Conference on Vitamin C, New York Academy of Sciences, Vol. 4, 98, 1987, pp. 153-167.*

The redox properties of ascorbic acid (vitamin C) and its dehydro and free radical (semidehydro and monodehydro) forms make the vitamin an important intracellular electron donor-recptor. There are, however, few studies to relate this activity to an essential role of the vitamin.

**Stimulation of NADH Oxidation by Soybean Plasma Membranes Isolated by Aqueous Two-Phase Partitioning From Homogenates Prepared in the Presence of 10 mM Ascorbate**

| Additions | Concentration | NADH-Oxidase (nmol/min/mg Protein) |
|---|---|---|
| None | | 15.7 |
| 2, 4-D | 10 μM* | 23.9 |
| Ascorbate | 1.7 mM | 20.4 |
| Ascorbate + 2,4-D | 1.7 mM + 10 μM* | 30.6 |
| Ascorbate radical§ | 1 mM | 24.4 |
| Ascorbate radical + 2, 4-D | 1mM + 10 μM* | 30.6 |

*Results with 1 μM 2,4-D were similar
§A mixture of equal parts of ascorbate and dehydroascorbate

Our interest in ascorbate-mediated electron transfer events has come from the discovery of a fixation-resistant NADH ferricyanide reductase associated with the trans half of the Golgi apparatus stack, transport vesicles, and the plasma membrane. This activity has subsequently been identified as an ascorbate free radical (mono- or semidehydro-ascorbate) oxidoreductase. Additionally, ascorbate has been reported as a constituent present in clathrin-coated vesicles, Golgi apparatus, chromaffin granules, and secretory vesicles, and has been implicated as important to the secretory process.

In this report, we summarize evidence for a role of ascorbate free radical as an electron acceptor in a redox system involving the NADH-free radical reductase that may reside uniquely in those cellular membranes (plasma membrane, coated vesicles, secretory vesicles, endocytic vacuoles) most often implicated in bulk translocations of cellular membranes and in membrane movements. The results are consistent with such a role as part of a mechanism to drive membrane translocations and for an involvement as an energy-producing enzyme (via generation of a proton gradient or of a membrane potential) in the generation of the force required for expression of cell motility. Additionally, transmembrane redox components may be important in the regulation of cell growth. Thus, a role for the ascorbate free radical as a natural electron acceptor in the growth process also must be considered....

While plasma membrane redox systems have long been implicated as important to regulation of growth processes, a role for ascorbate free radical as the electron acceptor has been less clear. Recent findings from plants, however, strongly support such a role.

With plasma membrane vesicles isolated by aqueous two-phase partition in the presence of ascorbate, some of the ascorbate trapped inside the vesicles is converted to ascorbate free radical. NADH oxidation by the plasma membrane vesicles is enhanced by isolation in the presence of ascorbate and a slight further stimulation is obtained from addition of either ascorbate or monodehydroascorbate (equal mixture of ascorbate and dehydroascorbate) to the reaction mixture....

Vitamin C in the DHA form is currently under investigation "as an electron acceptor in elongation growth in both animals and plants."

# ANTI-SCORBUTIC ACTIVITY OF L-ASCORBIC ACID & ESTER-C®

## Comparison of the Anti-Scorbutic Activity of L-Ascorbic Acid and Ester-C® in the Non-Ascorbic Synthesizing Osteogenic Disorder Shionogi (ODS) Rat

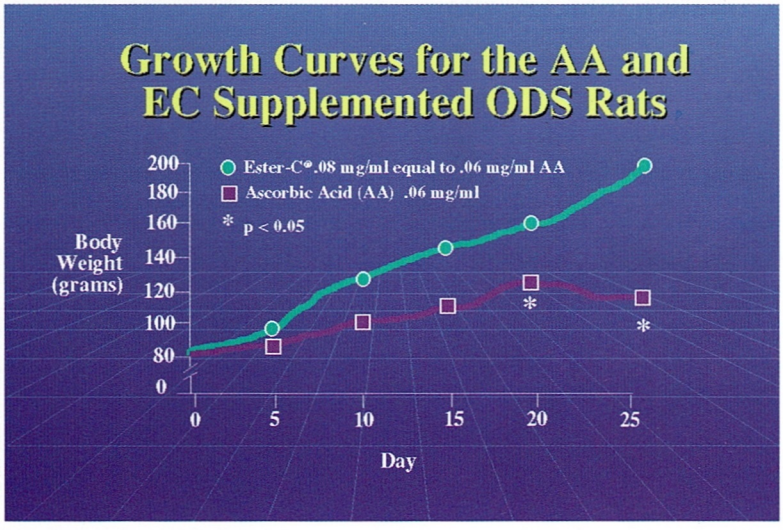
*By A.J. Verlangieri, Ph.D., Michael J. Fay and Anthony W. Bannon*
**Atherosclerosis Research Laboratories, Department of Pharmacology University of Mississippi, University, MS 38677**
***Excerpts from Life Sciences, Vol. 48, April 1991, pp.2275-2281***

The biological activity of the metabolites strongly differentiates Ester-C® ascorbate from conventional vitamin C. This study clearly shows that, based on ascorbate activity equivalents, "**Ester-C® is 4 to 5 times more potent and effective than ascorbic acid**."

The anti-scorbutic (anti-scurvy) potency of Ester-C®, a calcium ascorbate and calcium-threonate mixture, was compared with an ascorbic acid dose of equal ascorbate activity equivalents (AAE) for anti-scorbutic activity in the ODS rat....

Ester-C® rats had 3-4 times less, if any, scorbutic signs than ascorbic acid rats. The results clearly show that, based on ascorbate activity equivalents, Ester-C® has more available ascorbate activity / potency than ascorbic acid. This increased potency is believed to be due to the facilitated transport of AAE into the cell by the threonate (a normal *in vivo* metabolite of ascorbic acid) present in the Ester-C® product....

A normal metabolic product of L-ascorbic acid metabolism is calcium threonate. Bush and Verlangieri showed that Ester-C® administered at 10 mg/kg (AAE) versus ascorbic acid at AAE by gavage was ***absorbed more rapidly and excreted less rapidly*** in rats than ascorbic acid.... Maximum increase with calcium threonate was shown to be 177% greater than without calcium threonate....




The Ester-C® group had a significantly greater increase in body weight (125%) compared to the ascorbic acid group, which only had a 46% increase.... The difference in growth rate is most dramatic, with Ester-C® increasing body weight 3.5 times, a clear anti-scorbutic effect.... On the final day of the study, the ascorbic acid rats had 5 times more severe scorbutic signs than the Ester-C® rats.... It is clear from this study that, based on ascorbate activity equivalents, ***Ester-C® is 4-5 times more potent and effective than ascorbic acid*** in preventing scurvy.... Since the transport of ascorbic acid has been shown to depend, in part, on insulin, and may involve an energy-dependent process, threonate may act in concert with insulin at the cellular receptor.... Regardless of the mechanism of the increased potency of ascorbate activity when delivered as Ester-C® as compared to ascorbic acid, the increase is significant. The presence of ascorbic metabolites enhances or facilitates the utilization of ascorbate activity in this scorbutic model.

Can antioxidants that prevent low-density lipoprotein (LDL) oxidation inhibit atherosclerosis?

"We found that ascorbic acid itself cannot protect LDL against metal ion-dependent oxidation; rather, **AA is protective only after it has been oxidized to dehydro-L-ascorbic acid" or decomposition products such as threonate**.

"**Vitamin C more effectively prevents lipid peroxidation than vitamin E**... If LDL is stably modified by DHA or decomposition products... before it enters and is entrapped in the arterial wall, LDL may remain protected against atherogenic modification."

# PREVENTING OXIDATION OF LDL BY VITAMIN C & DHA

## Ascorbic Acid Oxidation Product(s) Protect Human Low Density Lipoprotein (LDL) Against Atherogenic Modification — Anti- Rather than Pro-oxidant Activity of Vitamin C In the Presence of Transition Metal Ions

*By Karen L. Retsky, Mason W. Freeman & Balz Frei*
From The Department of Nutrition, Harvard School of Public Health, Boston MA and the Department of Medicine, Massachusetts General Hospital, Boston MA

***Excerpts from The Journal of Biological Chemistry, Vol. 26a, No.2, Jan.15, 1993, pp.1304-1309 © 1993 by The American Society for Biochemistry and Molecular Biology, Inc.***

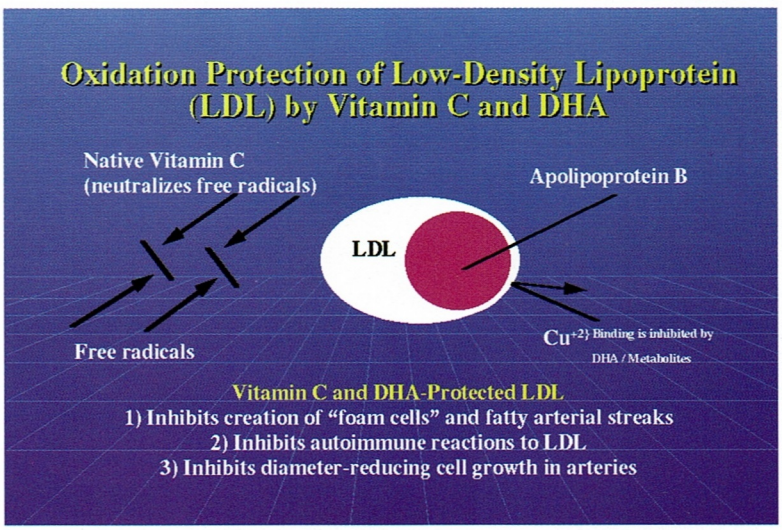
The oxidative modification of low density lipoprotein (LDL) has been proposed as an important causative event in the development of human atherosclerosis. As a corollary of this hypothesis, antioxidants that can prevent LDL oxidation may inhibit atherosclerosis.... Our data indicates that vitamin C protects LDL against atherogenic modification by two different mechanisms that may act in concert: (i) free radical scavenging by AA prevents aqueous oxidants from attacking and oxidizing LDL, and (ii) stable modification of LDL by DHA or decomposition product(s) thereof imparts increased resistance to metal ion-dependent oxidation. [These decomposition products include L-xylosone and L-threo-2-pentolusonic, L-xylonic, and L-threonic acids as well as L-threose]....

Oxidatively modified LDL (oxLDL), in contrast to native LDL, is taken up avidly by macrophages via scavenger receptor(s), leading to formation of lipid-laden foam cells, which are the hallmark of early atherosclerotic fatty streaks. OxLDL may promote atherosclerosis by other mechanisms as well, such as recruitment and retention of monocyte-macrophages in the arterial wall and cytotoxicity toward endothelial cells. Minimally oxidized LDL may also play an important role in atherogenisis, e.g. by stimulating expression of monocyte chemolactic peptide-1 and colony-stimulating factors in endothelial cells and smooth muscle cells. The "oxidative hypothesis of atherosclerosis" is supported by a number of *in vivo* findings. For example, oxLDL has been found in human atherosclerotic lesions, and increased titers of autoantibodies against oxLDL are present in sera of patients with carotid atherosclerosis....

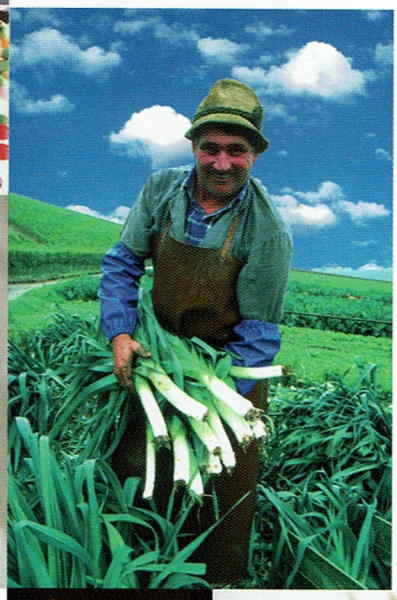
We demonstrated that vitamin C more effectively prevents lipid peroxidation than any other endogenous antioxidant in plasma and LDL, including vitamin E, which is the major lipid-soluble antioxidant in humans...

We found that AA itself cannot protect LDL against metal ion-dependent oxidation; rather, AA is protective only after it has been oxidized to dehydro-L-ascorbic acid (DHA). By a mechanism involving stable modification of LDL, DHA strongly prevents lipid peroxidation, shifts in anodic electrophoretic mobility, and uptake of LDL by macrophages....

Our observation that LDL is stably modified in the presence of DHA (resulting in increased resistance of LDL to oxidative modification) may have important implications for the prevention of human atherosclerosis by vitamin C. If LDL is stably modified by DHA or decomposition product(s) thereof in the circulation before it enters and is entrapped in the arterial wall, LDL may remain protected against atherogenic modification.


Oxidation Protection of Low-Density Lipoprotein (LDL) by Vitamin C and DHA
Native Vitamin C
(neutralizes free radicals)
Apolipoprotein B
LDL
Free radicals
Cu+2} Binding is inhibited by DHA / Metabolites
Vitamin C and DHA-Protected LDL
1) Inhibits creation of "foam cells" and fatty arterial streaks
2) Inhibits autoimmune reactions to LDL
3) Inhibits diameter-reducing cell growth in arteries

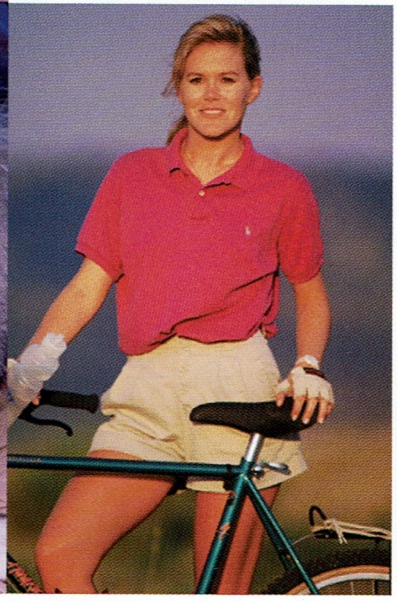
# FOUNDATIONS OF THE FUTURE



The future for **Ester-C®** ascorbates is bright. An ever-growing understanding of its metabolic mechanisms and antioxidant properties — as well as the unique and powerful roles its natural **metabolites** play — has opened a vista of potential new-product opportunities and applications research.

Promising early research and empirical observations have prompted aggressive scientific studies in the following disciplines:

- ***Food Technology***
- ***Cosmetic Science***
- ***Human Medicine***
- ***Dentistry***
- ***Veterinary Medicine***
- ***Agricultural Science***



**The Growing Family of Ester-C® Mineral Ascorbates**...Calcium, Magnesium, Zinc, Potassium, Sodium...

For more information contact:


**INTER-CAL CORPORATION**

*Exclusive Manufacturers of Patented Ester-C® Vitamin C Products*

WORLD HEADQUARTERS:
533 Madison Ave.
Prescott, Arizona 86301,
U.S.A.
Telephone (602) 445-8063
Fax (602) 778-7986

EUROPEAN OFFICE:
P.O.Box 2052
N-1522 Moss, Norway
Mobile Phone 47-30-84469
Telephone 47-69-27401
Fax 47-69-273400

## Inter-Cal Scientific Advisory Board

*Chairman*

**Jack Hegenauer, Ph.D.**
Research Director,
Inter-Cal Corporation

formerly, Associate Research Professor
Department of Biology
University of California San Diego

*Advisory Board Members*

**Anthony J. Verlangieri, Ph.D.**
Professor of Toxicology and Pharmacology
Department of Pharmacology
School of Pharmacy
University of Mississippi

Director,
Atherosclerosis Research Laboratories
University of Mississippi

**Steven J. Silverstein, D.M.D., M.P.H.**
Professor,
Department of Dental Public Health and Hygiene
School of Dentistry
University of California San Francisco

**L. Phillips Brown, D.V.M.**
President,
The Natural Connection
South Yarmouth, MA

**Howard F. Hunt, Ph.D.**
Professor Emeritus,
Department of Physical Education
University of California San Diego

President,
Life Management Groups, Inc.
La Jolla, CA

**Thomas B. Rice, Ph.D.**
Professor Emeritus,
Department of Health and Physical Education
San Diego Mesa College

Consultant,
Life Management Group, Inc.
La Jolla, CA

**Jeffrey S. Bland, Ph.D.**
Chief Executive Officer,
HealthComm, Inc.
Gig Harbor, WA

**Wayne Harris**
Aqueous Labs Inc.
Las Vegas, NV

**Michael E. Rosenbaum, M.D.**
Corte Madera, CA

**Inter-Cal**
CORPORATION

Exclusive manufacturers of Patented Ester-C® Mineral Ascorbates

January 31, 1996

Dear :

It was great talking with you. I appreciate your belief in and support of the use of Ester-C® in dogs. Since my study was first published, I have received a lot of favorable comments from people who supplement their pets' diets with Ester-C®.

I have enclosed copies of some recent articles on Ester-C®. If I can provide additional information or help in any way, please do no hesitate to call me at (520) 445-8063.

With best regards,
INTER-CAL CORPORATION

L. Phillips Brown, DVM
Technical Advisor

**HEADQUARTERS:** 533 MADISON AVENUE, PRESCOTT, ARIZONA 86301 U.S.A. TELEPHONE (520) 445-8063 FAX (520) 778-7986
**EUROPEAN OFFICE:** P.O. BOX 2052, N-1522 MOSS, NORWAY, TEL. 47-69-273400 FAX 47-69-273401, MOBILE 47-31-12365

SO UNIQUE,
IT'S PATENTED.
ESTER-C®
VITAMIN C
with CALCIUM
500 mg
100 tablets